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Virginia B. Elles

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Authority: Mr. J. W. Heim
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Date: 18 February 1948

SUBJECT: High Positive Pressure Breathing and Anoxia

OFFICE: Aero Medical Laboratory

SERIAL NO: MCREXD-696-104D Expenditure Order No. 696-61

A. PURPOSE:

1. To present the report entitled "Effects of High Positive Pressure Breathing and Anoxia", prepared by Drs. Chester Hyman, James P. Henry, J. Goodman, and J. P. Meehan, of the Department of Physiology, University of Southern California, Los Angeles, California.

B. FACTUAL DATA:

2. This report, attached as Exhibit 1, was submitted in fulfillment of Contract No. W-33-038-ac-14711.

C. CONCLUSIONS:

3. None.

D. RECOMMENDATIONS:

4. None.

Prepared by: J. W. WILSON, Ph.D.
Chief, Respiration Unit

Approved by: J. W. HEIM, Ph.D.
Chief, Physiology Branch

Approved by: A. P. GAGGE, Lt. Col., Ph.C.
Aero Medical Operations

Approved by: EDWARD J. KENDRICKS, Col., M.C.
Chief, Aero Medical Laboratory

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EXHIBIT I

EFFECTS OF HIGH POSITIVE PRESSURE BREATHING AND ANOXIA ON THE PERIPHERAL VASCULAR SYSTEM. By Chester Hyman, James P. Henry, J. Goodman and J. P. Meehan. Department of Physiology, University of Southern California, Los Angeles, California.

Summary

1. Apparatus and techniques are described to permit the maintenance of cats at ambient pressures below 50 mm. Hg with high pressure breathing and adequate counter-pressurization. Appendices IA, IB, and IC.

2. Data relative to the loss of fluid from the circulation of cats while pressure breathing with inadequate counter-pressurization are presented. The studies are extended to include pressure breathing of mixtures with low oxygen content to induce anoxemia. Such animals show a marked cardiovascular instability. Appendices IIA and IIB.

3. The effect of decreasing the venous oxygen tension on the rate of protein leakage from the circulation in the human arm is reported. Venous oxygen saturations below 15-20 per cent are required to cause measurable changes in protein leakage. Appendix III.

A study submitted as fulfillment of Contract No. W33-038-ac 14711, with the Aeromedical Laboratories, Engineering Division, Air Materiel Command, Dayton, Ohio.

4. Results with perfused tissues indicate a high tolerance of the capillary wall to both anoxemic and stagnant anoxia. Appendix IV.

5. The factors influencing venous pressure in a dependent limb are reviewed and some measurements reported. Appendix V.

6. Several methods for inducing polycythemia in experimental animals are compared. Appendix VI.

Introduction

Survival at extreme altitudes has become feasible through the development of the partial pressure suit and the use of high positive pressure respiration. The several conditions taken together constitute a novel environment for man and animals and present some unusual physiologic stresses. Counter-pressurization theoretically protects the subject against undue cardiovascular and mechanical disturbances which would result from the increased respiratory pressures required to provide him with an adequate oxygen supply. When the counter-pressurization is not complete, however, there is opportunity for significant decrease in the circulating blood volume due to congestion, pooling and filtration in the unprotected areas. Under these same conditions it is likely that the unprotected areas would suffer from hypoxia, which might in turn engender a capillary breakdown leading to further loss of fluid from the circulation. Such a situation would ultimately lead to disturbance of the normal circulatory dynamics and possibly the death of the animal.

The significance of these added stresses was assessed in the present studies by separating the several factors into independent systems. In each system the stress

was increased far beyond that anticipated and the physiologic responses noted. Since we chose to extend the experiments up to death as an end-point, it was desirable to conduct these studies on animals.

The findings indicate that relatively large areas of the body surface must be left unprotected before the fluid loss due to pressure breathing becomes significantly large. In addition, the capillaries of human and animal tissues are resistant to mild and moderate hypoxia, and definite changes in capillary permeability to protein occur only when the environment of the capillaries becomes extremel anoxic.

The practical and theoretical implications of partial pressure suits have been fully reviewed in previous reports from this laboratory. Henry et al. (1) have discussed the feasibility of partial pressure garments for maintenance of animals and men at altitudes in excess of 60,000 feet. Their work indicates that properly designed inelastic protective garments in conjunction with pressure bladders and "capstans," adequately protect against the aeroemphysema and the damage to lung tissue encountered in unprotected animals. They suggest that the counter-pressure exerted by the partial pressure suit be so regulated that the

shifts in body fluids are kept within reasonable limits and at the same time, make it possible to provide sufficient extra oxygen pressure to minimize the possibility of anoxia. Fluid loss into the inadequately protected areas has received special attention. Earlier studies were made on men while wearing the partial pressure suit and while wearing a protective vest only. These data clearly indicate the possibility of decreasing fluid loss by increasing the area protected. By adding protection over the limbs and abdomen as well as the thorax, fluid loss was decreased from about 9.2 cc./100 cc. of blood to about 4.7 cc./100 cc. of blood when the subjects were breathing at approximately the same pressure and for the same period of time. Since fluid loss in excess of 20 cc./100 cc. of blood is considered inimical to the cardiovascular stability of the individual, more extreme tests on humans seemed inadvisable. Fluid loss with more extreme pressures and with lesser degrees of protection was measured on experimental animals.

The earlier reports from this laboratory indicated that a subject at a simulated altitude of 55,000 feet, breathing at a pressure of 78 mm. Hg might experience an oxygen saturation as low as 88%. To achieve higher altitudes with no decrease in oxygen saturation would require higher

levels of pressure breathing which would in turn increase the tendency towards fluid loss from the circulation. On the other hand any further decrease in the oxygen saturation might, among other things, lead to a breakdown in the capillary wall and secondarily, to increased fluid loss from the circulation. It therefore became necessary to determine the relationship between the oxygen saturation of the blood and the permeability of the capillary walls. Other significant effects of anoxia would supervene and make for ineffectiveness of the subject; however, the present requirement was to assess with some accuracy the change of capillary permeability as a function of oxygen saturation without regard for central physiologic or psychologic effects. This problem was studied by two methods: first, the fluid loss and protein filtration into a congested human limb was measured under conditions of varying oxygen saturation of the venous blood; second, measurements were made of the rate of edema formation and associated changes in fluid balance in perfused animal limbs under conditions of varying oxygen partial pressure of the perfusion fluids.

It has been suggested that pressure breathing per se might lead to an instability of the cardiovascular system. Essex et al. (2) have shown that animals while pressure breathing can tolerate much less hemorrhage than

controls. It should be noted that the pressure breathing employed in these studies was not associated with protection and that under these conditions the hemodynamics of the thoracic circulation is highly abnormal. Protection during pressure breathing provides a nearly normal pressure system in the thoracic cage.

Six related studies were conducted under the present contract. The first study had to do with the construction of apparatus adequate for the maintenance of animals (cats) at ambient pressures below 50 mm. Hg. This included the design and construction of protective garments pressure breathing helmets, a suitable small decompression chamber and a system for the control and administration of appropriate pressure breathing to the animals. A second study was made of the fluid balance in cats subjected to pressure breathing with protection limited to the thorax and abdomen at sea level ambient pressures. An extension of this problem included pressure breathing of mixtures deficient in oxygen. The third problem was a re-examination of the fluid loss in a congested human arm as a function of the venous oxygen partial pressure. These experiments yielded data relative to the change in fluid loss and especially to the change in the protein loss from the

circulation at diminished oxygen levels. The fourth study made use of perfused isolated animal tissues for the estimation of the effects of diminished oxygen partial pressure on the rates of edema formation. These preparations make it possible to assess the effects of either stagnant or anoxemic anoxia on the vascular system of a mammalian preparation. A fifth study had to do with the normal mechanisms for the maintenance of venous pressure in dependent limbs. This study was undertaken to determine the extent to which venous congestion might result from immobilization of the limbs by either the protective garment itself, or by the positions imposed in aircraft. Finally, several of the techniques for the preparation of polycythemic animals were compared in order to obtain animals with a markedly increased oxygen transport capacity of the blood to be used in studies involving more extreme anoxia.

Methods and Results

1. Survival of animals at ambient pressures below 50 mm. Hg

The apparatus used in these studies is described in Appendices IA, IB and IC this report (cf. (3)). The apparatus included a system of pressure regulating valves

arranged to control inspiratory and expiratory pressures relative to the animal's ambient pressure (IA). An electronic timer control system (IB) permitted continuous variation in the duration of the inspiratory and/or expiratory phase of respiration in each cycle. A suitable steel, glass-topped decompression chamber equipped with proper connections for gas lines and electrical wiring was constructed (IC). The animals were afforded protection by the air-bladder vest combination described in Appendix IA and IC of the current report; the limbs and the head of the animals were given some protection by tight binding with Ace elastic bandages. EKG records were used throughout as a rough index of the condition of the animals.

Results: By the use of the apparatus and methods described above, we have been able to maintain cats at ambient pressures below 30 mm. Hg for significant periods of time. The data obtained are reported in Appendix IC.

There was in each case a marked distention of the exposed limbs and in addition, there were some signs of progressive deterioration of the heart. However, these cats when returned to normal atmospheric pressure quickly recovered a normal EKG, a normal respiratory pattern and a relatively low pressure showed a reversal of the swelling

of the exposed limbs. Insufficient fluid loss data suggest a lesser amount of filtration than anticipated.

2. Estimation of fluid loss in animals undergoing high pressure breathing.

Detailed results of these studies including a description of the apparatus used are given in Appendices IIA, IIB, IIC and IID of this report (cf. (3 & 3a)). In brief, cats were subjected to high pressure breathing with adequate counter-pressurization over the thorax and abdomen, and head and neck. Fluid loss was determined by changes in the hematocrit, hemoglobin, plasma protein and plasma dye concentrations; measurements on samples taken before, during and after the period of pressure breathing.

Results obtained with a modification of the apparatus, are reported in Appendix IID. The modification includes a lucite helmet to obviate the necessity for tracheal cannulation. (Appendix IIC of this report).

In addition, the pressure breathing system has been altered by replacing the small orificed valves with much larger electrically operated valves and by modifying the connections by installing tubing of dimensions appropriate to the required gas flow. This second modification minimizes the artifacts with regard to the respiratory pressures and rates required for the maintenance of animals.

first characterized by a normal whole blood volume with a subnormal plasma volume; the second phase showing a normal rate of increase in plasma volume with a further increase in whole blood volume.

Acknowledgments

The several studies accomplished under this contract were made possible by the close cooperation of all the staff of the Departments of Physiology and Aviation Medicine. Those immediately concerned with the execution of these experiments included Richard Bancroft, R. W. Berdan, R. Frankel David Gordon, Gerald Green, Byron Howells, Aaron Klain and Martha Mill. The professional staff included Doctors Joseph Goodman, James Henry, Chester Hyman and J. P. Meehan. Dr. James Henry and Dr. D. R. Drury served in a consultative and administrative capacity.

Discussion

The diversity of these studies makes it impossible to summarize the work in a few general conclusions. However, certain of the studies combine to confirm the general impression concerning the high tolerance of capillaries to anoxia. The results of the studies on the human arm and the studies with perfused tissues indicate that only extreme anoxemia

can alter the normal capillary permeability. The limiting venous oxygen levels (15-25%) reported in the former studies (Appendix III) are far below the level required for consciousness in the human, so that capillary damage (as indicated by protein leakage) would not supervene in a subject with sufficient oxygen for consciousness. These low levels were achieved by means of a prolonged congestion of a single limb in addition to exposures to diminished ambient pressure.

The extremely low venous oxygen levels (3.6 vol. per cent) noted in the cats during pressure breathing with air (Appendix IID) were due to some interference with normal circulation into the limbs of these animals. The protective garments used in these experiments did not include pressurization over the legs and may have constituted some sort of hindrance to flow in the extremities. In the group which failed to survive the experiment, the control venous oxygen averaged 2.5 vol. per cent compared with 4.2 vol. per cent in the surviving animals.

The studies with interrupted perfusions (Appendix IV) suggest that the transitory or short periods of congestion or stagnation are not sufficient stress to cause capillary damage. Periods of stagnation up to 15 minutes may be considered "safe."

Engineering Division
Memorandum Report No. MCREXD-696-104D
18 February 1948

In the design of protective garments every effort should be made to avoid features which might lead to congestion of the limbs to avoid the possibility of reducing the oxygen saturation in the restricted congested area below the critical level. It is unlikely that the partial pressure suit with its uniform pressurization over the surface of the limb could lead to such undesirable congestion.

Pressure breathing studies on human subjects have been made on un-anesthetized individuals who subjectively controlled the rate and pattern of respiration, while in the animal studies the respiration was a passive function with rate and pattern arbitrarily set. Further work would be required to ascertain the feasibility of using high pressure breathing on comatose or unconscious humans.

A small number of cats at sea level pressures were subjected to the same level of pressure breathing used for the maintenance of animals at extreme altitude (Appendix I). None of these controls survived. The actual fluid loss measured in one cat subjected to pressure breathing at diminished ambient pressure (15 cc./100 cc.) was far below the amount anticipated on the basis of the sea level pressure breathing studies. Though the number of animals involved was very small, the results suggest that at extreme altitude

the animal is in some way protected against the extreme pressure breathing. One possible mechanism of protection may be found in the aeroemphysema which develops at ambient pressures below 40 mm. Such a condition under the taut skin of the limbs might suffice to prevent significant fluid loss under conditions of high pressure breathing. Further studies with measurements of fluid loss would be required for solution of this problem.

Cardiovascular instability of the animals undergoing pressure breathing has been suggested in several studies. The results of our experiments (Appendix IID) indicate in addition, a diminished tolerance to anoxia. A significant number of animals have been shown to be incapable of surviving a diminished oxygen saturation during pressuring breathing. However, it should be pointed out that these animals had already suffered a fluid loss of about 10-15% of their circulating blood volume and were probably in a state comparable to hemorrhagic shock. In the light of newer concepts of shock (9), it is not difficult to understand the fatal effects of a superimposed anoxia.

The great reduction in venous pressure in a dependent limb during activity (Appendix V) is especially significant in considerations of fluid loss from the circulation.

Since the venous pressure in the vessels draining any region determines the effective hydrostatic pressure in the filtration area, abnormally high venous pressures would result in increased filtration pressure and consequently, in an increased loss of fluid from the circulation. The magnitude of this fluid loss has not yet been evaluated in this laboratory, although it has been shown (10) that continued erect posture with minimum movement is sufficient to induce hemo-concentration and increased plasma protein levels. Under the condition to be encountered by a pilot, it is not likely that the maximum venous pressure would be found in the foot since he performs continuous movements with his legs and he is usually seated. However, any immobilization of the dependent extremities, or any change in the posture of the pilot which would tend to increase the heart-to-foot distance should be avoided.

Of the several techniques attempted for the production of polycythemia in experimental animals, the intermittent exposure to diminished pressures seems to be the most useful (Appendix VI). Other techniques, including the use of cobalt and the use of continued blood transfusions (11) offer only slight advantage in final hematocrit attained, or in time required to establish the polycythemic state. The cobalt technique is attended by certain toxic manifestations

which probably make the animal abnormal for most studies, while the technique of continued transfusions may disturb the normal hemopoietic mechanisms and again render the animals abnormal (12).

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Appendicies

Appendix I

A. Pressure Breathing System for Experimental Animals. Chester Hyman and Aaron Klain.

B. Continuously Variable Biphastic Interval Timer. Aaron Klain.

C. Survival of Cats at Ambient Pressures Below 40 Mm. Hg. Chester Hyman, Aaron Klain and R. Bancroft.

Appendix II

A. Determination of Fluid Loss from Experimental Animals During Pressure Breathing. Joseph Goodman, Chester Hyman and David Gordon.

B. The effect of High Pressure Breathing on the Escape of Fluid from the Circulation of Experimental Animals. Chester Hyman and Joseph Goodman.

C. Modifications in Animal Pressure Breathing Apparatus. Chester Hyman, Aaron Klain and Martha Mill.

D. Combined Effects of Pressure Breathing and Anoxia on Fluid Loss from the Circulation of Experimental Animals. Chester Hyman, Joseph Goodman and Rosalie Frankel.

Appendix III Capillary Permeability in Relation to Acute Anoxia and to Venous Oxygen Saturation. James Henry, Joseph Goodman, J. P. Meehan and Rosalie Frankel.

Appendix IV Method for Perfusion of Isolated Rat Tissues. Chester Hyman and Ralph W. Berdan.

Appendix V Effects of Temperature and Exercise on Venous Pressure in the Foot when in the Erect Position. James P. Henry.

Appendix VI Production of Polycythemia in Rabbits by Anoxia and Cobalt. Joseph Goodman and Byron Howells.

Engineering Division
Memorandum Report No. MCREXD-696-104D
18 February 1948

Appendix IA

Pressure Breathing System for Experimental Animals

Chester Hyman and Aaron Klain

The equipment used by Henry et al. (1) for the pressure breathing of animals at altitude has been modified to permit greater flexibility in the control of the several pressures involved. The chief advantage of the present system for experimental work is the fact that the pressure regulatory system may be located at sea level pressures, permitting ease of control at all times independent of the pressure to which the animal is exposed.

Fig. 1 is a photograph of the apparatus as we have set it up; Fig. 2 is a schematic diagram of the connections. R_i, R_e, and R_v are Bastian-Blessing, type 2500 butane regulators. These are connected on their high pressure side to a source of oxygen regulated to 5 p.s.i. gauge pressure and on their low pressure side to independent out-put lines. The left-hand detail in Fig. 2A indicates a "reference pressure port" on the spring loading side of the regulator diaphragm. This port is connected in each case to a reference pressure line (Line Ref. of Fig. 2) connected to the decompression chamber. The several out-put lines are

connected to differential manometers, M_i, M_e and M_v, which measure the out-put pressures relative to the reference pressure.

The out-put from R_i connects directly to one side of an electrically operated four-way valve, which in turn connects to a 'T' at the opening of the tracheal cannula. From R_e, the out-put line leads to the loading side of a Linde mask exhalation valve, which is mounted in an air-tight case (cf. right detail of Fig. 2A). The second half of the four-way valve connects the Exhalation port of the Linde Capsule to the tracheal cannula. The dumping port of the Linde Capsule is connected by a low pressure line (L.P. of Fig. 2) to the vacuum pump. The out-put from R_v may be connected directly to the bladder of the protective vest worn by the experimental animal.

S_i, S_e and S_v are safety valves, Fisher Governor Co., Type 289 U, which are adjusted to permit the blow-off of excess pressures into the low pressure line, L. P.

In operation, the pressures in the several lines are set to the required levels with the animal decompression chamber at "sea level." The pump is then started and the chamber evacuated. As the pressure in the chamber drops, there is a corresponding drop in pressure in the reference

line and consequently, on the loading side of the Butane regulators. Conditions are thus automatically adjusted to maintain the out-put pressures at a constant differential above the reference chamber pressure. As the reference pressure decreases, the pressure in the flowing lines can decrease correspondingly; however, the pressure between the regulator R_e and the Linde Valve, or the pressure between R and the static vest, would tend to remain constant, since the gas trapped between the regulator and the dead end could not escape nor flow back through the regulator. This would result in an increase in the relative pressures. The use of the safety relief valves (S_1, S_e and S_v) obviates this difficulty, permitting the trapped gas to flow across into the low pressure line when its pressure becomes excessive.

The operation of the four-way valve is such that the tracheal cannula is alternately connected to the inspiratory out-put line and to the Linde valve loaded to the desired expiratory pressure. The pressure at the cannula rises to the set inspiratory pressure during one phase of the respiratory cycles and falls towards the set expiratory pressure during the second phase. The use of a 'T' tube close to the cannula makes it possible to reduce the dead air space to a minimum.

Frequency of respiration, as well as the duration of the individual phase of respiration, is controlled either by a sprocket driven by a telechron motor, or by the more easily varied electronic timer described in Appendix II of this report.

(1) J. P. Henry, D. R. Drury, E. Movitt. On the development of aeroemphysema at 65,000 ft. simulated altitude in animals. Memorandum Report on Emergency Pressure Suit, Serial No. TSEAA-660-100, 5 May 1946, Headquarters, Air Materiel Command, Engineering Division

Engineering Division
Memorandum Report No. MCREXD-696-104D
18 February 1948

Appendix IB

Continuously Variable Biphasic Interval Timer

Aaron Klain

The complete regulation of the rate of respiration of experimental animals requires the independent control of two periodically successive periods of time. This problem has been solved in this laboratory by the construction of the electronic instrument to be described.

In its essentials, the apparatus consists of two independently controlled relaxation oscillator circuits to provide triggering action, each of which is coupled to the grid of thyratron. Two thyratrons are arranged to form a controlled "flip-flop" circuit, which in turn controls a relay. The supplied voltage for the condenser charge is not applied to the triggering circuit until the appropriate thyratron has been fired.

The action of the complete circuit is as follows. Fig. 3 is a complete schematic circuit diagram of the timer. Between the cathod of T_1 and ground there is a series R-C circuit. When T_1 fires, C_1 begins to charge through R_1. When the potential of C_1 rises to the firing voltage of the VR tube, T_3, T_3 fires, discharging C_1 through the 5000 ohm

resistor, the resulting voltage pulse being sufficient to trigger T_2, the grid of which is connected to the positive side of the aforementioned resistor, with consequent extinction of T_1. As T_2 fires, the relay in its cathode circuit, operating a D.P.D.T. switch, closes. One side of the switch controls the solenoid operated valve in the respiratory supply lines, while the other side selects C_1 to short to ground, thus preparing it for service on its next charging cycle. To the cathode of T_2 is connected a second R-C circuit, a duplicate of that associated with T_1. The action is identical, the charging of C_2 resulting in the firing of T_4 and the transfer of current from T_2 to T_1. The relay opens as T_2 extinguishes, discharged C_2 completely and switches the respiratory valves as desired.

It was found necessary to discharge C_1 and C_2 completely after each action to prevent residual charge from affecting the independence of timing of both parts of the cycle.

The dwell of each part of the cycle is determined by the value of R_1 for one and R_2 for the other. As used at present, a nomograph has been constructed, from which the appropriate settings of R_1 and R_2 can be read once the frequency (cycles per minute) and the ratio of inspiration time to expiration time has been set.

It is our intention to revise the present instrument to enable the operator to set the latter quantities directly on two control dials without requiring the use of the nomograph.

Engineering Division
Memorandum Report No. MCREXD-696-104D
18 February 1948

Appendix IC

Survival of Cats at Ambient Pressures below 40 mm. Hg

Chester Hyman and Aaron Klain

Apparatus:

Fig. 4 is a photograph and Fig. 5 is a diagram of the small steel decompression chamber used in these studies. The chamber is equipped with ports for vacuum line, air bleeds, lines to bring gases under appropriate pressures from the regulator system (cf. Appendix IA) to the animal, as well as a nine-place electric terminal block for carrying through EKG and other electric lines. A 3/4" glass plate is sealed to the milled top edge of the chamber with a little plasticine to effect closure.

The vacuum line connects the chamber with an "Ingersoll-Rand, 5 and 5 x 3½ Vacuum Pump," with both cylinders connected in parallel. With the chamber properly sealed and with no leak or animal, this system is capable of reaching an absolute pressure of about 15 mm. Hg in about 2 minutes. With an animal in the chamber and with the requisite leak equal to the exhaled volume of gas, we have never been able to achieve pressures below 21 mm. Hg in a similar 2 minute interval.

Methods:

Cats were prepared by tracheotomy and were afforded protection by the air-bladder vest combination described in Appendix IIA and IIC. The neck and head of the animal was tightly wrapped in ace bandages; the four limbs and the tail were exposed directly to the external pressures.

In each case sub-dermal EKG electrodes were inserted into each limb. EKG records were taken at sea level with normal breathing, at sea level with pressure breathing and at intervals during the flight. The gross characteristic of the EKG at any time were taken as an index of the condition of the animal and dictated proper adjustments of altitude, respiratory pressures or respiratory rates.

The individual animals were placed in the chamber and connected with the pressure breathing system set at levels of approximately 60 mm. Hg inspiratory pressure, 25 mm. Hg expiratory pressure, with the bladder of the vest coupled to the pressure line between the tracheal cannula and the four-way valve. The glass plate was then sealed across the top of the chamber and the ascent begun.

The chamber was "leveled off" at a simulated altitude of about 40,000 ft. and the respiratory pressures were adjusted upwards to give a mean pressure near 100 mm. Hg. The ascent was then continued until the pressure in the

chamber reached a minimum. The time for ascent rarely exceeded 10 minutes. This pressure was maintained for 30 minutes, or until the EKG showed drastic changes which could not be corrected by alterations in respiratory pressures or rates. The pressure in the chamber was then allowed to increase until the EKG became more nearly normal.

In one case, a sample of femoral blood was taken before the cat was exposed to decompression and pressure breathing, while a second sample was taken immediately after descent. The second sample showed an increase of more than 15% in hematocrit.

In the earlier experiments attempts were made to maintain the pressure in the protective bladder independent of the respiratory pressures, at some constant level between the inspiratory and expiratory value. This procedure soon proved to be untenable and the bladder pressure thereafter was coupled to the lung pressure by cross-connection. The most suitable respiratory rate proved to be about 60 cycles per minute, with about 2/3 of the period in inspiration and 1/3 in expiration. In order to maintain a normal animal at ambient pressures below 50 mm. Hg with the apparatus used, inspiratory pressures of about 150 mm. Hg and expiratory pressures of about 60 to 80 mm. Hg were required. In the

original apparatus, the small orifices of the valves and tubing used exercised an extreme hindrance to the flow of gases and it is likely that the respiratory rates required and pressures measured represent artifacts due to this technical inadequacy. A further technical source of error should be noted. As will be pointed out in another section, a significant percentage of the animals used in this and the following studies were probably suffering from a variety of undiagnosed conditions which resulted in abnormal blood pictures.

Seventeen cats were exposed to pressures of about 30 mm. Hg in nineteen experiments. Of this number, twelve of the animals survived the exposure. In general, the animals which survived were somewhat larger than those which died. (See Table I). In several cases the death of the animal was traced to causes unrelated to the exposure to altitude: viz., in two cases the cannulation was totally ineffective, leading to strangulation; in another case autopsy revealed a unilateral hydronephrosis. Three animals were exposed to the same levels of pressure breathing with equivalent protection at sea level. All died in less than 30 minutes.

Insufficient data were accumulated to make definite conclusions concerning the loss of fluid from the circulation of animals under these conditions. In one case, a single

blood sample taken immediately after descent from altitude had a hematocrit value of 40% as compared with a pre-flight control value of 31.2%, indicating a fluid loss of about 15 cc./100 cc. of blood.

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Appendix I

Table I

Survival of Cats at Extreme Altitude

	No. of Animals	Average Weight	Minimum Pres. (mm. Hg)	Time at Alt. (min.	Mean Rep. Pr.
Survivals	7	6#, 12 oz.	26.3 (22.3-33)	17.7 (2-30)	120
Deaths	5	5#, 2 oz.	31.9 (22.3-58)	16.3 (4.5-30)	130
Unrelated Deaths	4	5#, 2 oz.	30.4 (22.5-38)	12.6 (2.5-20)	120
Sea level Controls*	3	7#, 0 oz.	Sea level	22.0 (9-31)	127

* All sea level control animals died. The times noted refer to the period of exposure to high pressure breathing for these control animals.

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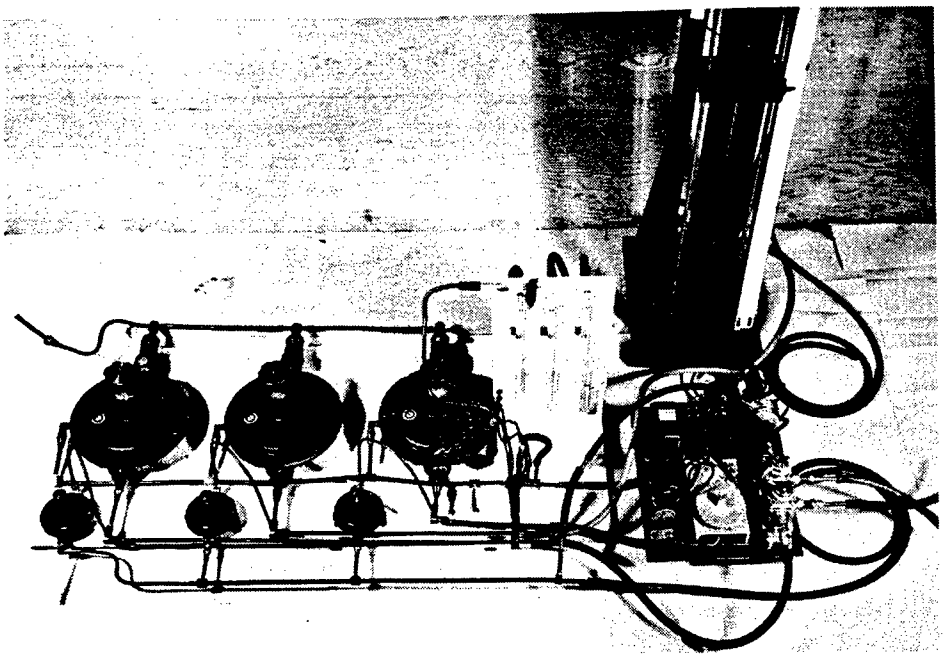


Figure 1. (Appendix I)

Photograph of pressure-breathing system, showing regulators, timing-cam, solenoid valves, etc.

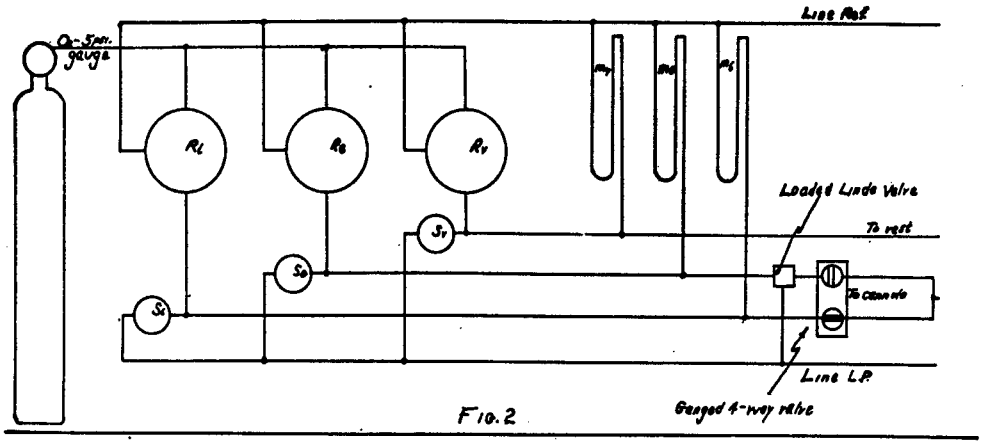
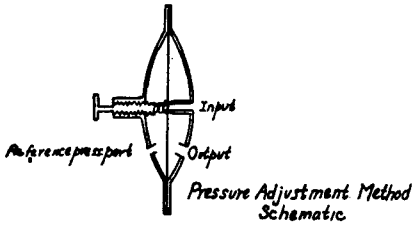


Fig. 2



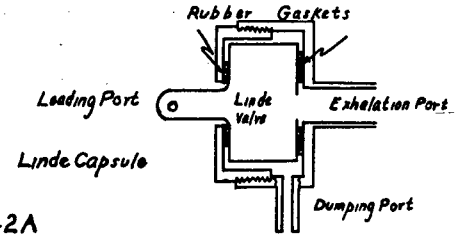


Fig. 2A

Figures 2 and 2a (Appendix I)

Diagram of pressure-breathing system. Details of regulator and of "Linde Capsule". For description see text, Appendix IA.

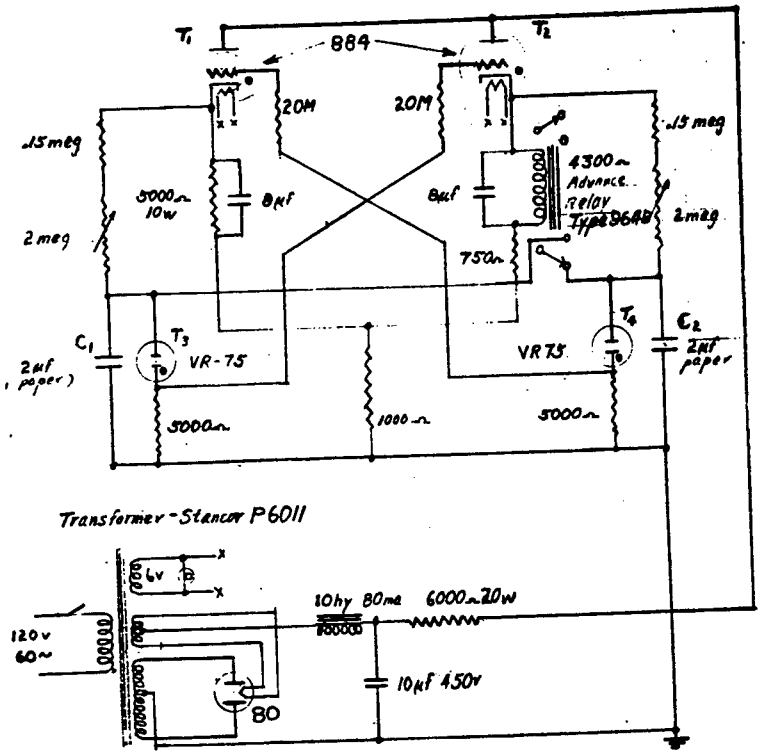


Figure 3. (Appendix I)

Circuit diagram for electronic timer. For description see text, Appendix IB.

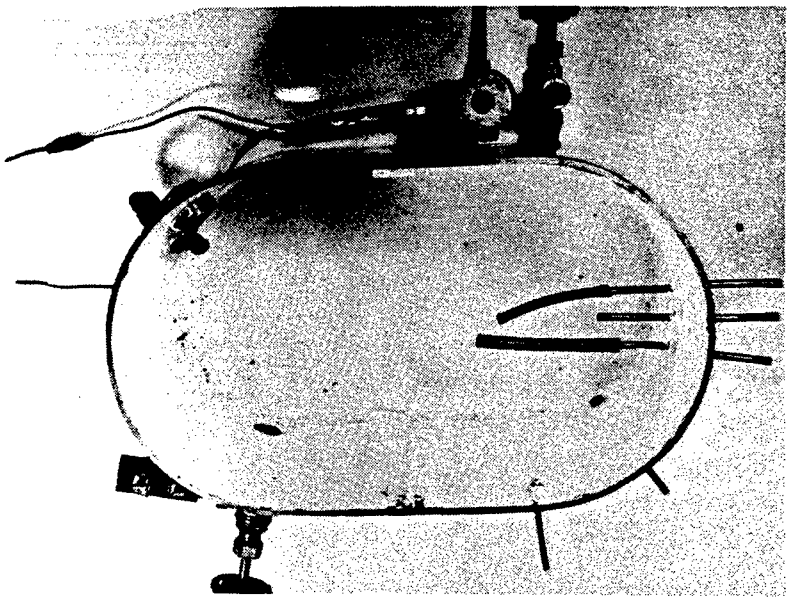
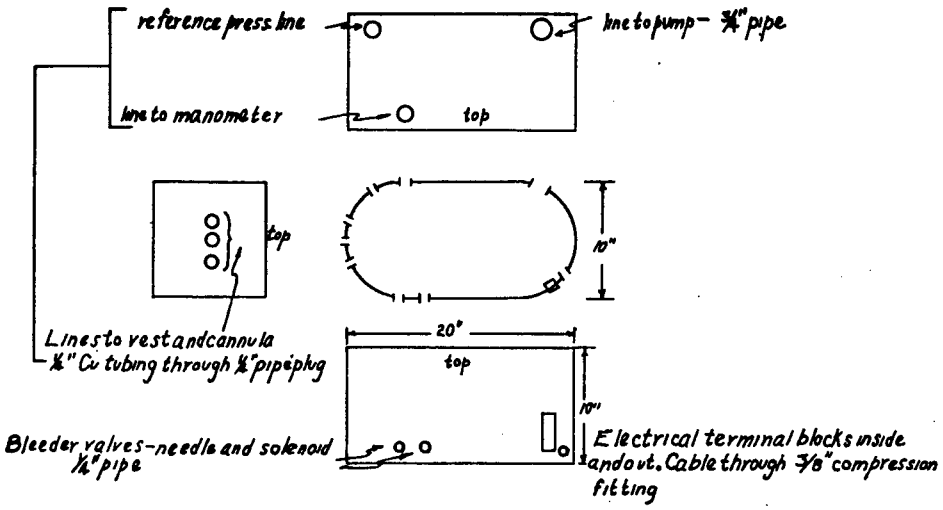


Figure 4. (Appendix I)

Photograph of animal decompression chamber. Closure of the top is accomplished by a 3/4" thick plate glass, sealed with a rubber gasket.



Arrangement of Connections into Animal Chamber

Fig 5

Figure 5. (Appendix I)

Diagram of animal decompression chamber. For details see Appendix IC.

Engineering Division
Memorandum Report No. MCREXD-696-104D
18 February 1948

Appendix IIA

Determination of Fluid Loss from Experimental Animals during Pressure Breathing

Joseph Goodman, Chester Hyman and David Gordon

Apparatus:

The apparatus used was that described in Appendices IA and IB of this report. In addition, the animal was protected by a pneumatic-bladder and a non-distensible vest which covered the entire abdominal and thoracic region. (See Appendix IIC). The principle of this protective covering is the same as that on which the partial pressure suit is based; it affords a method for supplying a well regulated counter-pressure against the abnormally high intrapulmonary pressures and permits high pressure breathing without injury to the lungs.

Methods:

a. The preparation: Cats under nembutal anaesthesia were prepared by the insertion of a tracheal cannula, the outer end of which terminated in two arms forming a 'T.'

The large blood vessels in the femoral region were exposed and the protective bladder and vest were fitted on the animal. The neck and head were tightly bound in Ace

elastic bandages. Sub-dermal EKG electrodes (made from hypodermic needles) were placed in each of the unprotected limbs to give the three normal EKG leads and a grounding connection.

In several cases a suitable Statham strain gage was inserted in the tracheal cannula pressure line in order to obtain a measure of the actual intrapulmonary pressures.

In several cases the out-put from the dumping port of the Linde Capsule was connected to a suitable flow meter in order to obtain an approximate index of the ventilation rate.

b. Sampling techniques: Fluid shifts were determined from three sets of data: Hematocrit, hemoglobin and dilution of an injected non-diffusable dye. These measurements were made on samples of blood drawn directly from the femoral artery. For each sample 1 ml. of blood was taken into a syringe containing 1.6% Sodium Oxalate. The amount of oxalate and of blood was determined gravimetrically and were approximately in a ratio of 1 to 5. After thorough mixing, ca. 0.8 cc. was used in a Wintrobe Hematocrit tube. The remainder, ca. 0.3 to 0.4 cc., was utilized for the hemoglobin determinations with the colorimetric acid hematin technique. The hematocrit values were determined directly

from the portion in the Wintrobe tubes. The supernatant plasma in the hematocrit tubes was removed by pipette. 0.2 ml. of this plasma was diluted with 1 ml. of 0.9% saline solution. The dye concentration was determined with a Beckman quartz spectrophotometer. All data have been corrected for dilution of oxalate and saline.

c. General procedure: Immediately after completing the preparation, a single control sample was taken and soon thereafter, about 0.1 mg. T-1824 per pound body weight of cat was injected into the femoral vein. At precise intervals after the dye injection, further samples were taken. About 50 minutes after injection of the dye, the tracheal cannula was connected to the pressure control system. The pressures were set at approximately 60 mm. Hg for inspiration, 30 mm. Hg for expiration, and the vest pressure was held at either 45 mm. Hg or was coupled to the tracheal pressures. Respiratory rate was held at approximately 60 c.p.m., with about 2/3 of each cycle in inspiration, 1/3 in expiration.

At intervals during the period of pressure breathing, further samples were taken. After 30 to 70 minutes, the animal was disconnected from the pressure system and samples were taken during the subsequent 90 minutes.

The state of the animal was at all times judged by observation of the EKG records and appropriate

adjustments of pressures and rates were made to maintain the EKG near normal.

Results:

Fig. 1 presents the data obtained in a single experiment. During the first hour under anaesthesia, the hematocrit and the hemoglobin concentration of the blood increased steadily. The magnitude of this change was small compared with the subsequent increase. After pressure breathing was begun, there was at first no apparent increase in the concentration of erythrocytes, but this was followed by a definite and rapid rise in both hematocrit and hemoglobin concentration. Both these indices of erythrocyte concentration continued to rise as long as pressure breathing was continued (50 minutes in this experiment), although there will presumably be some limit to this process of hemoconcentration which would be reached in pressure breathing of longer duration, i.e. when the tissue pressure has attained sufficiently large values. After pressure breathing was stopped, the hematocrit and hemoglobin concentration decreased rapidly for about 40 minutes, and then leveled off at values only slightly above those exhibited prior to pressure breathing.

The curve of dye concentration shows the continuous decrease (characteristic of the normal disappearance curve) interrupted by a gradual rise during pressure breathing.

Engineering Division
Memorandum Report No. MCREXD-696-104D
18 February 1948

These indices are in qualitative agreement on the loss of fluid from the circulation. The implication of the dye dilution curve is that the fluid lost is probably of a lower protein concentration than whole plasma, since it is believed that the Dye T-1824 is normally coupled to the plasma proteins. Quantitative evaluation of pressure breathing as an agency for inducing fluid loss must await further studies.

Engineering Division
Memorandum Report No. MCREXD-696-104D
18 February 1948

Appendix IIB

The Effect of High Pressure Breathing on the Escape of Fluid from the Circulation of Experimental Animals

Chester Hyman and Joseph Goodman

Studies by Henry et al. (1) on fluid loss from the circulation of human subjects during pressure breathing suggested a quantitative relation between the levels of pressure breathing and the amount of fluid loss when conditions of protection by counter-pressure were held constant. The great variation in the results and the limited fluid loss tolerable in human experiments prompted us to repeat these studies on experimental animals with levels of pressure breathing beyond those used in the earlier work. Such animal experiments have added advantages of complete control over the respiratory pressures and pattern, ease of sampling and post-mortem examinations for possible correlative factors.

Apparatus:

The apparatus has been previously described, (Appendicies IA and IB) (2).

Fluid shifts were determined from four sets of data detailed in Appendix IIA. In addition, plasma protein concentrations were determined from the specific gravity of the remaining supernatant fluid by the falling drop technique. The remaining details of technique corresponded to those used in

Appendix IIA.

Results:

The results obtained in 21 successful experiments are summarized in Table I. The table is divided into sections according to the pressures employed; the last line in each section gives the mean values for the several pertinent measures. The final section of the table presents data relative to animals giving aberrant results.

In column 3 are listed the percentage fluid loss from the circulation based on changes in hematocrit value. These data were obtained by dividing the maximum hematocrit obtained during the period of pressure breathing into the average of the pre-pressure breathing values, and subtracting the result (multiplied by 100) from 100. Column 4 presents the corresponding data obtained from the hemoglobin values. Agreement is generally good between these data.

Column 5 gives a qualitative estimate of the leakage of protein from the circulation, based upon comparison of hematocrit and plasma protein concentration curves during the cours of the experiment. A + sign indicates marked protein leakage, while a - indicates no appreciable leakage and a ± refers to some intermediate condition.

The remaining columns of the table refer to measurements made on samples taken in the pre-pressure breathing

control period. Thus, column 6 is the average of several plasma protein concentration determinations made before pressure breathing was begun. Similarly, columns 7 and 9 are the average of the pre-pressure breathing hemoglobin and hematocrit values respectively. Data in columns 8 and 10 were obtained by dividing the appropriate plasma protein value (column 6) by the corresponding hemoglobin value to obtain column 8 and by the hematocrit value to obtain column 10. We propose to refer to these values as the "absolute hemoglobin" and "absolute hematocrit."

Except for the data obtained at 90 mm. Hg inspiratory pressure, there is at least a qualitative increase in fluid loss with increased respiratory pressure. These results are in substantial agreement with the results of Henry et al. (1) as indicated in Table II.

The data obtained at 90 mm. Hg are probably entirely unacceptable for several reasons. At this higher pressure there was a shifting of the protective vest to overlay the axillary and femoral regions interfering with the circulation in the exposed limbs. In both of the animals used at this pressure samples of blood taken from the femoral artery were obtained with difficulty, confirming the suggested interference with the circulation. In none of the other animals was such

difficulty experienced. This decreased circulation would tend to lower the fluid loss under the conditions of our experiment so that these data must be discarded.

The data presented in section E of the table are distinguished from the remainder of the data by the following inherent differences:

(1) These animals all showed marked or intermediate leakage of protein, whereas the other animals showed no or little protein leakage.

(2) Both the "absolute hemoglobin" and "absolute hematocrit" indices were significantly higher in this group than in the remainder of the animals. It should be noted that these indices are based on measurements taken <u>before</u> the animals are subjected to pressure breathing and hence, represent an appraisal independent of the animals' response to the experimental procedure.

The values of the dye determinations were corrected according to Noble and Gregersen's equation - $D_t = P_o/P_t . D_c$ (4), where D_t is observed dye concentration at time t, P_o is plasma protein concentration before dye was injected, P_t is plasma protein concentration at time t. D_c equals theoretical dye concentration if plasma volume were unchanged. Parallel corrections were made with the hematocrit and the hemoglobin

data. The logarithms of these corrected values were then plotted against time. Examples of the dye disappearance curves after correction on the basis of the several parameters are given on Plate No. 1.

The curves showed an abnormally long mixing time of 40 minutes for cats, compared with 20 minutes or less for man as reported by Gregersen. Control animals and experimental animals gave identical curves so that there is no evidence that pressure breathing alters the fundamental dye disappearance curve. This is nicely verified by all three parameters, viz., the plasma protein, hematocrit and hemoglobin (Figs. 2 and 3).

A qualitative estimate of the protein leakage may be obtained from the curves presented on Plate 2. Here the per cent increase from the pre-pressure breathing value has been plotted for hematocrit, hemoglobin and plasma protein data. If there is close correspondence among the three curves it may be assumed that there was minimal protein leakage; where the plasma protein shows significantly less increase than the hematocrit or hemoglobin, it may be assumed that there was significant protein leakage. An example of good protein retention is given by Fig. 5; whereas Fig. 6 presents data indicative of great protein leakage.

Discussion:

We have been able to obtain in animals fair corroboration of the findings of Henry et al. covering the relationship between the loss of fluid from the circulation and the level of pressure breathing imposed upon an animal. It should be noted that our animals were only partially protected, i.e., their limbs were totally unprotected and hence, there was an opportunity for the escape of fluid from the increased pressure in the circulatory system into the relatively normal pressures of the tissues in the unprotected areas. There is fair quantitative agreement when these data are compared with corresponding data for man. However, the variability encountered in these studies, as measured by standard deviations, is about as great as that noted in man and so it appears unlikely that much greater accuracy can be gained from the use of animals. In addition, the type of protective bladder and vest now in use interferes with the normal circulation at the higher pressures and thus, limits the range over which experimentation is possible.

In the majority of the animals there was no evidence of appreciable protein loss into the filtrate. This would suggest that under the conditions of the experiment there was no appreciable change in the perviousness of the vascular wall,

and that the fluid loss represents a simple filtration from the circulation as a consequence of the inequality of pressures. Our evidence does not permit us to state whether or not the increased intravascular pressures are sufficient to cause degrees of vascular distension incompatible with absolutely unchanged vascular permeability, but there is certainly no great alteration.

In those cases where protein leakage was marked there was a corresponding increase in the amount of fluid loss, which is consistent with the breakdown of the osmotic mechanism for the retention of fluid in the circulation. These animals also differed from the normals in that their "absolute hemoglobin and hematocrit" indices were abnormally high. These indices represent a measure of the concentration of circulating hemoglobin relative to the more stable plasma protein level instead of the labile plasma volume. It is noteworthy that those animals with high indices (low concentration of hemoglobin) showed the greatest protein leakage and fluid loss. It would seem that the vascular system in these animals is rendered permeable to proteins by the techniques employed. This susceptibility may be related to an inherent pathology which is reflected by the altered indices. Whatever their

exact significance, these indices offer a technique for the prediction of animals which will show during pressure breathing undue protein and fluid loss.

The dye disappearance curves when properly corrected for fluid shifts indicate no measurable deviation from control animals. That is, we can find no evidence that pressure breathing alters the mechanism of dye disappearance from the circulation in spite of the gross effects of this same procedure on the shift of fluid from the circulation. This finding is similar to the results reported by Noble and Gregersen (4) who find no shift in the properly corrected dye disappearance curves during severe shock in man, even in the face of massive transfusion.

Engineering Division
Memorandum Report No. MCREXD-696-104D
18 February 1948

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1. J. P. Henry, I. Hendrickson, E. Movitt and J. P. Meehan. CAM, CMR No. 452, July 1945.

2. Chester Hyman and Aaron Klain. Appendix I to First Bi-monthly Report on this Contract.

3. Aaron Klain. Appendix II to First Bi-monthly Report, this Contract.

4. R. P. Noble and M. I. Gregersen. J. Clin. Invest., Vol. 25: 158, 1946.

Engineering Division
Memorandum Report No. MCREXD-696-104D
18 February 1948

Appendix IIC

Modifications in Animal Pressure Breathing Apparatus

Chester Hyman, Aaron Klain and Martha Mill

The apparatus used for pressure breathing studies on animals had been modified to obviate certain technical inadequacies in the original design. The changes included increase in the size of the electrically operated valves and the connecting tubing used, modifications in the design of the protective air bladder and jacket and the design of a new helmet.

The "ganged four-way valve" referred to in the original report has been replaced by two alternately operated Electrimatic Solenoid valves, Model No. 2660. All of the lines carrying gas from the regulators to and from the helmet on the animal have been replaced by rubber tubing with 3/4" internal diameter. The same size tubing is used to cross connect between the respiratory circuit and the pressurized protective air bladder. The "Linde Capsule" has been rebuilt to present a smaller resistance to out-flow of gas. The "body" is constructed of a short section of lucite tubing, ca. 3" in diameter. One end of the tube is plugged with a piece of 3/8" lucite, turned to permit a press fit

and cemented in place. Into this sheet there are cemented three tubes for connection with a manometer, and outlet for expiratory gases and an inlet for inspiratory gases M, E, and I of Fig. 7, respectively. To the other end of the "body" there is tied and cemented one end of a somewhat conical tube of air-tight vinylized nylon cloth. The seal between the helmet and the animal is accomplished by carefully placing this air-tight skirt between the outer surface of the air bladder and the jacket. Since pressure in the bladder is approximately equal to the pressure in the helmet at all times, the seal is satisfactory.

Certain changes in the jacket and air bladder were necessary for use in conjunction with this helmet. The pattern in Fig. 8 approximates the type used in the construction of these garments. The air bladder is made of two thicknesses of vinylized nylon cloth cut with appropriate openings for all four limbs and open along the back (A to B of Fig. 8) for ease in donning. The stitched edges are made air-tight by sealing with a rubber cement, and gas is conducted into the bladder through an aluminum brushing, bolted and cemented to the external layer only. The jacket design closely follows the same pattern, except that it is made of a single layer of Berger cloth and is provided with a tongue

and eyelets alone the opening on the dorsal side of the animal to permit lacing. In addition, a suitable strap and buckle system is sewed to the head end of the jacket to permit tighter fastening around the neck. A second belt system runs forward from the head end of the tongue of the jacket and passes over the helmet to buckle to the front end of the ventral surface of the jacket, thus preventing the helmet from creeping forward off the head of the animal.

Test animals have been subjected to pressure breathing levels up to 180 mm. Hg inspiratory with only minimal leakage using this new apparatus.

Engineering Division
Memorandum Report No. MCREXD-696-104D
18 February 1948

Appendix IID

Combined Effects of High Pressure Breathing and Anoxia on Fluid Loss from the Circulation of Experimental Animals

Chester Hyman, J. Goodman and Rosalie Frankel

In addition to the data reported in Appendix IIB, experiments have been performed with the revised apparatus. The results generally confirmed those reported above. Variability was quite marked and was, to some extent, associated with the state of the animal as measured by the "absolute hemoglobin index" discussed in the previous report. In eighteen cases the animals were exposed to the combined stress of anoxia and pressure breathing. In these studies, cats were subjected to pressure breathing with compressed air for a control period of 30-60 minutes, followed by a similar period of pressure breathing with a gas mixture containing less than 20% oxygen with all other conditions of the pressure breathing maintained constant. Pressure breathing was continued with air for a final control period. Samples were taken during the several parts of the experiment for the determination of the fluid loss and in addition, for measurement of the venous oxygen saturation. The data are summarized in Table III.

The oxygen saturations measured during the control pressure breathing period were far below normal level. This condition might have resulted from the anaesthetic used (nembutal), or it might be considered an indication of stagnation of blood in the unprotected limbs. In these studies the respiratory pattern was the same as that used in the earlier work, with the single modification of a decrease in rate from 60 to 30 cycles per minute. The fact that animals could be maintained at a rate more nearly normal is taken as evidence of the adequacy of the pressure breathing system.

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Appendix II

Table I

1	2	3	4	5	6	7	8	9	10
	Wgt. in	Hort. Fluid	Hgb. Fluid			Control			
Date	Kg.	Loss	Loss	Prot. Leak.	Plas. Prot.	Hgb.	PP/Hgb.	Hort.	PP/Hort.
				A - 44 mm. Hg.					
9/30	2.5	5.7			6.09			43.1	14
				B - 50 mm. Hg.					
10/9	2.55	8.4	8.8	±	5.27	11.9	44	42.8	12
10/14	1.81	2.5	7.7		6.26	10.4	62	39.5	16
10/21	3.26	10.3	11.6	-	6.97	12.2	57	43.7	16
Averages		7.1 ± 3.3	9.4 ± 1.7				54		15
				C - 60 mm. Hg.					
7/10	2.86	14.0	16.4						
7/16	2.38	15.1	12.9						
7/19	3.11	13.4	15.6						
7/24	3.09	14.0	13.9						
8/6	-	13.3	12.8	±	5.97	10.6	56	39.1	15
8/9	2.78	12.5	13.6	-	6.15	11.1	55	38.4	16
8/21	2.5	14.8	11.0	-	6.75			42.7	16
8/28	2.27	10.5	10.7						
Averages		13.4 ± 1.4	13.4 ± 1.9				55		16
				D - 90 mm. Hg.					
9/11	3.77	7.8	9.3		7.08	10.8	65		
9/18	3.42	4.7	4.5						
				E - Aberrant Results					
50 mm. Hg.									
10/2	2.75	33.9	30.4	+	6.93	8.8	78	32.4	21
10/23	4.70	35.8	34.2	+	7.76	8.3	93	30.5	25
8/7	3.43	23.2	22.4	+	7.16	11.0	65	38.5	19
60 mm. Hg.									
8/13	3.28	34.8	35.0	+	6.81	9.4	72	35.4	19
8/20	-	22.3	19.1	±	6.74	9.9	68	37.7	18
9/25	3.17	20.3	23.6	±	6.67	9.3	72	35.4	19
Averages		28.4	27.5				75		20

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Appendix II

Table II

Fluid Loss with Pressure Breathing

Respiratory Pressure	50 mm. Hg		60 mm. Hg	
	Hort.	Hg.	Hort.	Hgb.
Present Study	7.1±3.3	9.4±1.7	13.4±1.4	13.4±1.9
Henry et al.	8.5±1.5	9.5±1.4		

Appendix II

Table III

Fluid Loss in Pressure Breathing Anoxic Cats

	Number of Animals	% Fluid Loss cc./100 cc.	Absolute Hb. Index	Venous O_2 Control Vol. %	Venous O_2 Exptl. Vol. %	Exposure to Anoxia Min.
With no anoxia	12	10.5	--	--	--	--
Successful	7	11.1	--	--	--	--
Failure	5	7.5*	--	--	--	--
With anoxia	18	15.4				34
Successful	10	16.5	46.4**	4.2	3.8	33.8
Failure	8	14.2	49.6	2.5	0.1	34.5

* Data available on only one animal

** In these combined groups, animals with absolute hb Index greater than 48.4 had an average fluid loss of 25.8%, while those with an Index less than 48.4 had an average fluid loss of 16.9%.

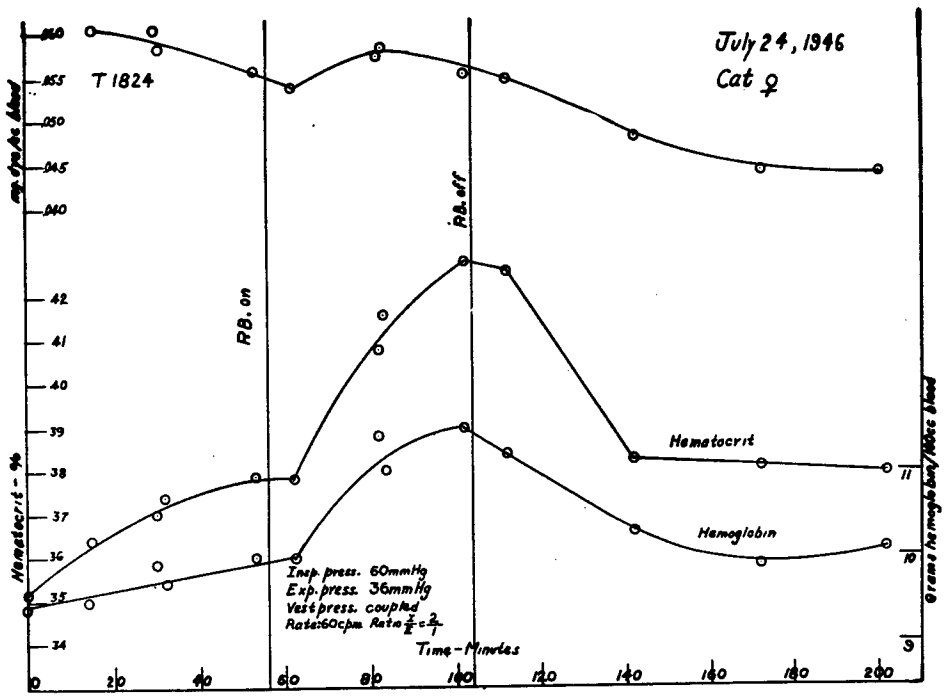


Figure 1. (Appendix II)

Hemoglobin, hematocrit and dye dilution data from cat during pressure breathing. See Appendix IIA.

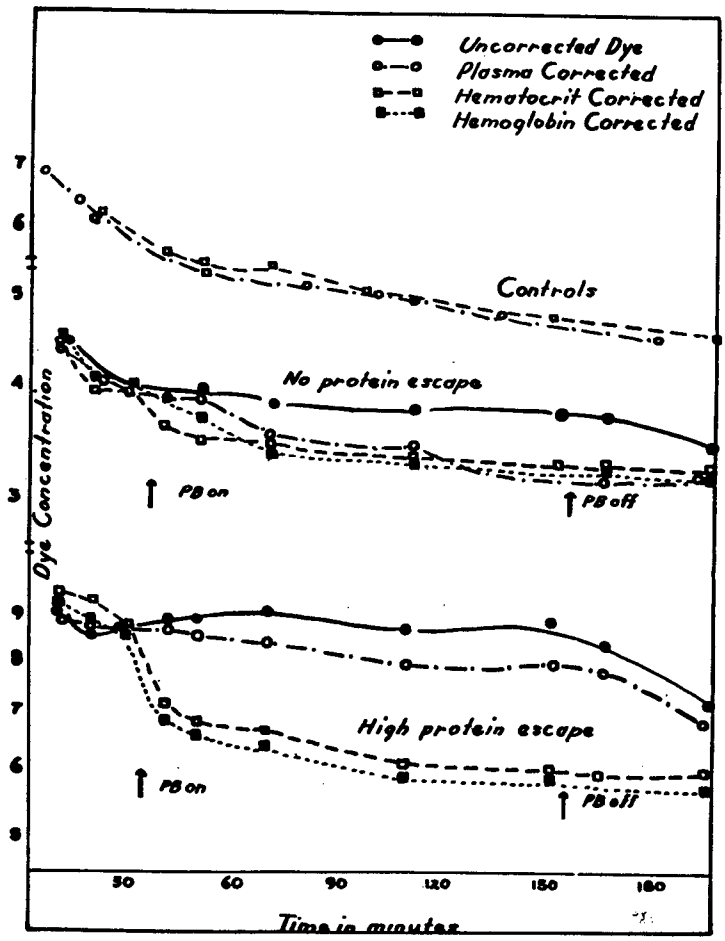


Plate I

Figures 2, 3, and 4 (Appendix II)

Dye-dilution data, for control cats, pressure-breathing animals with no protein escape and pressure breathing cats with high protein escape respectively. See Appendix IIB.

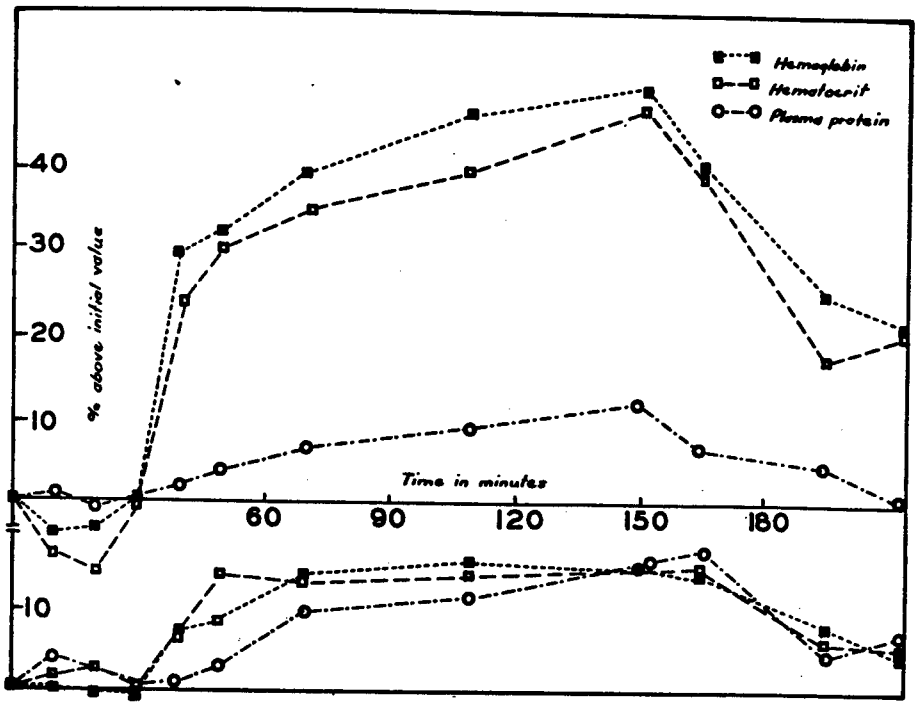


Plate II

Figure 5. (lower) and 6. (above)
(Appendix II)

Percent change in hemoglobin, hematocrit and plasma protein levels during pressure breathing. Figure 5 represents good protein retention. Figure 6 represents high protein loss.

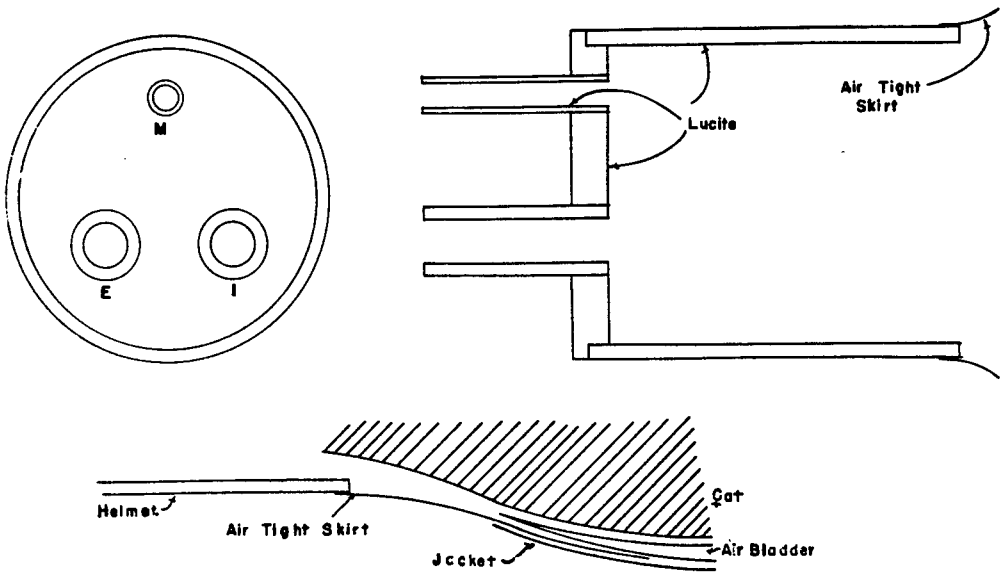
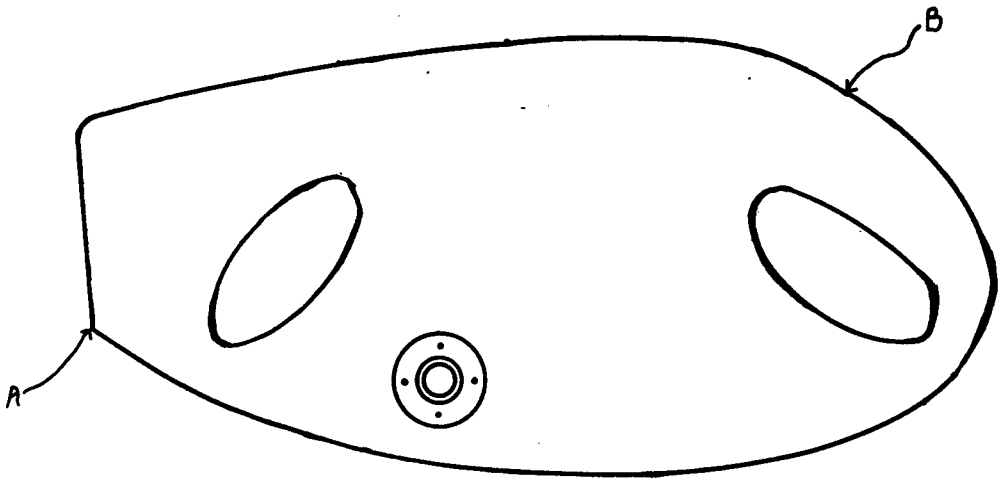


FIG. 7

Figure 7. (Appendix II)

Details of pressure breathing helmet and helmet-to-cat seal. For discussion see Appendix IIC.



PRESSURE BLADDER

Figure 8. (Appendix II)

Pattern for air-tight bladder. Seam from A, over upper left corner to B is left open. See Appendix IIC.

Engineering Division
Memorandum Report No. MCREXD-696-104D
18 February 1948

Appendix III

Capillary Permeability in Relation to Acute Anoxia and to Venous Oxygen Saturation

J. Henry, J. Goodman, J. Meehan and R. Frankel

Introduction

It has often been assumed that moderate degrees of hypoxia render the capillaries abnormally permeable to fluid and protein. During the course of work concerned with methods of insuring pilot survival at altitudes above 45,000 ft., it became desirable to know the degree and duration of anoxic anoxia that could be tolerated before permeability changes of the capillary bed occurred. The studies of Landis (1) have demonstrated that complete ischemic anoxia of 3 minutes duration will cause temporary damage to the capillaries of the frog's mesentery and result in abnormal permeability to fluid and protein. Pochin (2) working with the rabbit's ear has found that complete occlusion of the circulation for two hours will lead to demonstrable edema following re-establishment of the circulation. He also showed that 16-18 hours of occlusion resulted in edema fluid containing approximately 5 gms. per cent of protein. Calvin (3) has found that during the terminal stages of fatal asphyxia there is evidence of a marked loss of protein and of the protein attached dye T 1824 from the capillary bed.

These experiments involved very intense anoxia and on turning to work done under less severe conditions, the results are seen to be less conclusive. Thus, Calvin (3), and also Hopps and Lewis (4), found no change in the rate of filtration of plasma protein and of the dye T 1824 in the course of severe but non-fatal anoxia. On the other hand, Maurer (5) and Warren and Drinker (6) have shown that in the dog the flow of cervical and lung lymph increases during severe but non-fatal anoxic anoxia. However, the percentage of protein in this lymph decreases with the increased flow and the results do not unequivocally demonstrate an increase in capillary permeability as a result of the acute anoxia. Observations concerning the influence of anoxia have been made in humans. McMichael and Morris (7) have used nitrogen-oxygen gas mixtures of an oxygen percentage (9.5%) that would induce approximately 65% arterial hemoglobin saturation. They found no increase in the rate of swelling of the congested arm. Their results suggested that the permeability of the arm capillaries to fluid is unchanged by anoxia. However, the technique employed did not give them any information on the concentration of the protein in the filtrate and their report is in abstract form only. Another study is that by Stead and Warren (8), who have estimated the protein content of the edema fluid in two emphysematous patients whose arterial

oxygen saturation was 50-60%. They found no significant change from the normal figure of 0.25 gms. per cent or less, and concluded that this degree of chronic anoxia had not increased the permeability of the capillaries of the limbs. In an earlier report (9) the authors found no significant change in fluid or protein loss from the capillaries of the forearm when under the influence of acute anoxia.* The following paper confirms these results and extends them to an extimation of the approximate oxygen tension at which a significant increase in protein filtration from the capillary bed occurs.

Method of Approach

The best measure of the permeability of the capillary wall would be a direct determination of the concentration of

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* A systematic error which does not, however, invalidate the conclusions drawn has been detected in the calculation of the protein data presented in this report to the CAM of the OSRD, and summarized in the Fed. Proc. 1946, 5, '44. The corrected figures for the calculated protein in the filtrate are 1.5 gms. 1.5 gms. per cent when anoxic and 1.2 gms. 0.5 gms. when at sea level.

protein in the capillary filtrate (10). A significant increase in this concentration over the normal, near zero (0.2-0.3 gms. per cent), values found in the limb may be regarded as evidence of a change in the permeability of the wall (1, 8). Failing a direct determination, an estimate of this measure was made by Landis et al. (11) in their experiments, using various degrees of venous congestion supplied by the blood pressure cuff. The congestion cuff causes a marked slowing of blood flow without stopping it when the venous pressure imposed by it (60 or 80 mm. Hg) is less than the arterial blood pressure. The increased filtration pressure of the slowly flowing blood results in a loss of fluid into the tissues. Comparison of the hematocrit values shown by the blood in the median antecubital vein of the congested arm with that in the uncongested control arm permits an estimation of the fluid loss in cc. per 100 cc. of the blood flowing through the congested arm. These investigators have devised a formula by which they can calculate the percentage of protein in the capillary filtrate from observations on the relative changes in hematocrit values and plasma protein levels in the blood from the control and congested arms. In such studies on the human arm, they have observed a calculated protein in the capillary filtrate at a congestion

pressure of 80 mm. Hg of approximately 1.5 gms. per cent. This procedure produces the double effect of an increase of the hydrostatic pressure in the capillaries and an ischemic anoxia due to slowing of the blood flow through the vessels. It has been pointed out (11) that if it were possible to be sure that the high pressure in the congested capillary bed was not directly responsible for this abnormally high content of protein in the filtrate, then the experiment would provide evidence of the effect of ischemic anoxia on capillary permeability. Kunkel, Stead and Weiss (12), and more recently Allen et al. (13), have shown that adrenalin injections will lead to a marked increase in the amount of blood flowing through a limb. If this increase in blood flow is enough to effectively eliminate the ischemic anoxia resulting from a congestive cuff, then it should be possible to eliminate the protein loss in a congested arm by increasing the blood flow through it by the use of adrenalin. If the protein loss is eliminated in spite of the use of cuff pressures of 80 mm. Hg, then it can be assumed that such pressures have little effect on the permeability of the blood vessels. Another method of dissociating the effects of pressure from those of anoxia would be to decrease the venous oxygen tension while keeping the capillary pressure constant. This could be

accomplished by exposing the subject at a cuff pressure at which the protein in the filtrate was at the upper limit of the normal values to a lowered alveolar oxygen tension. Reduction of the arterial oxygen saturation to 60-70% would cause a considerable fall in the mean capillary oxygen tension. This fall should increase the protein in the capillary filtrate if permeability is significantly affected by changes in oxygen tension. Since the oxygen tension is lowest at the venous end of the capillary, the oxygen content of the venous blood should be a measure of the oxygen tension in that part of the capillary bed which is exposed to the most severe anoxia. If the content of protein in the filtrate is a measure of the permeability of the capillary wall and if this permeability is affected by the oxygen tension, then it should be possible to demonstrate a correlation between the oxygen content of the venous blood from the congested arm and the concentration of protein in the capillary filtrate.

Methods

The subjects employed were chosen for their familiarity with experimental procedures and the majority of the tests were performed on only three persons. These were in good health and of an active habitus. The two subjects employed for the altitude studies showed no gross vasomotor disturbnaces with acute anoxia anoxia. There was no significant

change in blood pressure, pulse or respiration rate with reduction of the arterial oxygen saturation to values of the order of 60% for periods of 30 minutes. It was hoped that by employing repeated tests on a few well trained subjects it would be possible to decrease the inherent differences between various subjects and the variations due to fluctuations of subject response.

Adrenalin hypertension was obtained by the intramuscular administration to the recumbent subjects of 6/10 cc. of 1/500 adrenalin in peanut oil, followed in 15 minutes by 1/10 cc. doses of 1/1000 adrenalin subcutaneously in sufficient number to maintain the blood pressure 20-40 mm. Hg above the normal resting level for the subject. In the subjects employed, this meant that the systolic blood pressure was maintained at 140-160 mm. Hg for the 30 minutes of 80 mm. Hg cuff application.

During the altitude tests each subject rested for 30 minutes before putting on the cuff at 60 mm. Hg for 30 minutes. The temperature of the chamber in which the tests were conducted was uniform. However, no readings of skin temperature were made, and it is thought that some of the variations in the results noted may have been due to minor changes in the condition of the skin and deep circulation from day to day as the tests progressed. The same arm was always

used for congestion in order to decrease experimental variations.

Bloods were collected with 0.1 cc. of liquid heparin in 10 cc. syringes and immediately set up in duplicate in Wintrobe hematocrit tubes. Duplicate hemoglobins were estimated, using the acid hematin technique. R.B.C. volume was obtained by correcting the hematocrit readings by a factor of 8.5% for fluid trapped between the cells (14). Plasma protein concentrations were determined in duplicate, using the falling drop technique. Venous oxygen content and saturations together with venous carbon dioxide contents were determined in duplicate by Van Slyke blood gas analysis in a number of the tests using 60 mm. Hg congestion and anoxia and in all those using 80 mm. Hg congestion with adrenalin hypertension.

Anoxia involving 55-65% saturation of the arterial blood was obtained by ascent in a decompression chamber to a simulated altitude of 19,000 to 20,000 ft. The subjects were relaxed and dozed in a recumbent posture on a couch. Respiratory rate, blood pressure and pulse were checked every 5 minutes, and the oxygen saturation followed throughout by a Millikan compensated oximeter. Contamination of the altitude chamber atmosphere by oxygen was avoided by using an oxygen mask with an exhalation valve which was connected to a tube venting directly into the chamber exhaust. The oxygen saturation,

rarely higher than 65%, was maintained at 60% and frequently fell to 55%.

Calculations

The equations used to determine the per cent protein in the filtrate were those developed by Landis et al. (10). As they have pointed out (10, 11), these equations become less accurate with lower degrees of fluid and protein loss. In order to assess the variability introduced by these calculations of protein in the filtrate, the standard errors of the hematocrit and protein readings were determined. These were found by making repeated measurements of the same sample. They were ± 0.2% for the hematocrits and ± 0.06 gms. per cent for the plasma proteins. These values were inserted into the equations, and the maximum positive and negative deviations were calculated for each set of experimental data. In Fig. 1 such values obtained for each experiment have been plotted against the product of the hematocrit and protein differences, yielding a parameter which indicates in part, at least, the variability to be anticipated in the protein in the filtrate. If the product $(Ht_2-Ht_1)(Pr_2-Pr_1)$ is small, a condition which would be associated either with a low fluid loss and usually small protein leakage or both, then the variability is great. High values show an increased accuracy. Inspection of the

Figure also reveals a negative bias which is largest at the lowest values of protein change and fluid loss.

In a considerable proportion of the experiments the calculations yield negative values for protein concentration in the filtrate. Such negative values might result from a failure to take account of unrecognized physiological variables. Alternatively, in view of the large random error and more especially of the negative bias introduced by the equation, the values obtained could be the result of the mathematical skewing of the formula with consequent negative bias noted above.

Results

I. Adrenalin Hypertension

In Table I are presented the results of 8 cuff experiments on 3 subjects using a cuff at 80 mm. Hg for 30 minutes. There is fair agreement between the mean values for the fluid loss as calculated from the changes in the hematocrit 13.4 ± 1.7 cc./100 cc., and those calculated from the hemoglobin values i.e., 12.8 ± 1.8 cc./100 cc. This suggests that no significant changes in cell volume occur as a result of the stasis induced by the cuff experiment, for such changes would affect only the hematocrit values (10). The marked individual variations from experiment to experiment may be in part attributed to changes in rate of forearm blood flow due

to differences in temperature, activity and other variables. Attempts to obtain more reliable figures by using an indwelling needle in the radial artery (15) as a source of the control blood led to no improvement. However, it is of interest that in their original series of 5 experiments, using 80 mm. Hg cuff pressure, Landis et al. obtained a mean fluid loss figure of 15.0 cc./100. This compares favorably with the above values.

In Table II are seen the data obtained from the same number of experiments on the same 3 subjects performed in the same way except that a hypertension of 140-160 mm. Hg was maintained throughout the ½-hour period of cuff application. The fluid loss figures show good concordance between the hemoglobin and hematocrit values. The values of 6.0 $\pm$ 0.6 and 6.6 $\pm$ 0.8 cc./100 cc. respectively, are almost exactly one-half those obtained for the normal controls. This is interesting in view of the observation by Allen et al. (12), that adrenalin administration can double blood flow through the uncongested arm. For if the blood flow rate were doubled and other factors, such as tissue pressure, remained relatively unchanged, then the fluid loss per 100 cc. blood should be decreased in the same proportion.

It might be expected that the A-V O_2 difference would be greatly decreased if such a change in flow rate had

occurred. In practice the mean venous oxygen saturation rose from 18.0% to 30.5%. It is of interest that numbness, tingling and eventual loss of sensation to light touch developed in the congested arms in the controls. Such symptoms never occurred at 80 mm. Hg cuff pressure in the cases with adrenalin hypertension. This suggests that the blood flow under these conditions is adequate to maintain normal metabolism but that the reduced flow in the absence of hypertension is insufficient to do so. It may be noted that Griffith et al. (16) point out that it is probable that adrenalin has no significant effect on tissue oxygen utilization. Therefore the rise in oxygen saturation following its use is probably not due to a decrease in tissue metabolism.

The change in the average protein percentage in the filtrate from 0.8 $\pm$ 0.5 gms. per cent to a mean value of - 1.0 $\pm$ 0.3 gms. per cent as a result of the use of adrenalin is significant. It is not probable that the percentage of protein in the filtrate would change with changes in the rate of flow of the plasma past the filtering area. It would seem likely that the increase in mean venous oxygen saturation has had some part to play in the decrease in protein in the filtrate. The negative mean value for the protein figures can be explained by the bias of the expression for protein in the filtrate to

negative values by the random errors of sampling and estimation. As had been pointed out under calculations (Fig. 1), this error is greater in the case of the adrenalin experiments in which the fluid loss is small than in those where these changes are larger, as in the control experiments. It is not certain, however, whether there may not also be some undetermined variable which may account in part for the high negative values found in the adrenalin experiments.

The object of the adrenalin tests was to decrease the ischemic anoxia while keeping the capillary pressure constant. Since the protein in the filtrate decreased under these circumstances, the possibility that the high intracapillary pressure was responsible for the increased filtration of protein in the 80 mm. Hg cuff without adrenalin can be considered small. This leaves ischemic anoxia as the most probable factor responsible for the permeability changes.

II. Anoxia

In Table III and Iv are presented a series of 23 control sea level 60 mm. Hg cuffs on 7 subjects and 12 altitude experiments using 2 subjects. The mean sea level fluid loss of 7.6 cc./100 cc. $\pm$ 0.5 cc. as calculated from the hematocrit values was again close enough to that calculated from the hemoglobins of 8.2 cc./100 cc. $\pm$ 1.1 cc. to suggest that there is no significant change in red cell volume during the stasis induced by the cuff.

The mean venous oxygen saturation during the altitude experiments was 26.4%. The arterial oxygen saturation was maintained at 55-65% throughout the period of cuff application. The oxygen tension along the entire capillary was therefore much less than its normal value. On the other hand, in the subjects employed repeated checks of systolic blood pressure during the course of the cuff application at altitude showed no significant change. The absence of a significant change in the mean fluid loss when using anoxia (7.9 cc./100 cc. at sea level and 6.3 cc./100 cc. at altitude) contrasts with the marked change found in the experiments with adrenalin. It suggests that the pressure in the capillaries and the rate of blood flow through the forearm was not significantly affected during these latter tests. The changes found in the percentage of protein in the filtrate can therefore be attributed to the reduction in oxygen tension of the blood in the vessels. The difference between the mean value of 1.6 $\pm$ 0.4 gms. per cent obtained during the tests at altitude when contrasted with that of 0.4 $\pm$ 0.3 gms. per cent for the controls represents a definite increase in protein in the filtrate as a result of the change in venous oxygen saturation from the mean of 26.4% at sea level to 11.4% at altitude.

In Table V the evidence obtained from the two sets

of experiments is summarized. When the data from the altitude work is considered in conjunction with the evidence obtained in the adrenalin studies, a picture is obtained which suggests that anoxia has some effect upon capillary permeability in the forearm when the oxygen saturation falls below the general value of 25%.*

In Fig. 2 the values for protein in the filtrate are plotted against those for oxygen saturation in the venous blood. Inspection reveals a definite upward trend of these protein values at oxygen saturations of less than 25%. Curve A, Fig. 2, was obtained by adding to each determination of protein in the filtrate the positive deviations as calculated for Fig. 1,

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* Although venous oxygen determinations were not made for all of the 60 mm. cuff experiments (7 of 23 controls and 6 of 12 altitude experiments), the arterial oxygen saturation was reduced by the same amount in all cases. It is therefore assumed that the average venous oxygen saturation of those samples in which this measurement was not made would have been of the same order as the determined values.

plotting the resulting points and fitting the curve to them. Curve B was plotted in a similar manner using the negative deviations. They have no quantitative significance, but represent an attempt to indicate qualitatively the skewing and relative accuracy of the data over the range of venous oxygen saturations considered. The convergence of those curves towards the higher values of protein in the filtrate is an expression of the lower random error at the higher values for protein and fluid loss. The marked negative bias of the error, especially in the determinations with higher values of oxygen saturation, probably accounts in large measure for those negative values observed. The essential feature portrayed is the increase in the protein in the filtrate with oxygen saturation values of less than 20-30%. This is clearly shown on inspection of the data and can be confirmed by the above mathematical considerations.

Discussion

The foregoing results suggest that is is necessary for the oxygen tension in the capillary bed of the human arm to drop to values below 15-25 mm. Hg, corresponding to an oxygen saturation of 15-25%, or an oxygen content of 4-6 vols. per cent in those with a normal hemoglobin content, before any significant changes in permeability to protein can be expected.

However, if the oxygen tension falls below this value, there is good evidence that the anoxia is then of sufficient severity to increase capillary permeability even when it is of short duration. It is of interest that even during decompensation the oxygen content in blood from the anticubital vein of caridac patients only rarely falls to such values (17). Nor are such values met in pneumonia, except in moribund cases (18). In the cases of emphysema with cardiac failure described by Stead and Warren (8), in which in spite of an arterial saturation of 50-60% there was no increase in protein in the edema fluid, the venous oxygen content was not recorded. It is possible that in spite of the severe anoxic anoxia, the venous O_2 saturation was not below the critical level of 15-25%. One reason for making this assumption is the fact that such patients often have polycythemia and their blood can therefore yield more oxygen before reaching any particular degree of desaturation than can the blood of those with a normal hemoglobin.

In severe anemia the reverse of the above mentioned condition occurs, and subjects with hemoglobin concentration values of 20% or less show very low venous oxygen saturation values of the order of 20% (19). Strauss and Fox (20) have pointed out that the tendency to water retention on administration of sodium salts to those with severe anemia was not

due to a low plasma protein level or to an increased venous pressure. Although endocrine factors (21) and the effect of changes in renal blood flow (22) must first be considered in assessing the cause of this water retention, it is possible that a part of the edema sometimes noted in severe anemia may be due to increased capillary permeability as a result of anemic anoxia. It is of interest in this connection to note the indidence of edema in cases of erythroblastosis foetalis occurring in utero (23). For in such cases, in addition to the severe anemic anoxia present as a result of red cell destruction, there is a considerable anoxic anoxia as a consequency of the incomplete oxygenation of the fetal blood by the placenta.

Aub and Cunningham (24) have shown that in irreversible traumatic shock the venous oxygen content may fall as low as 3-4 vol. per cent, representing saturations of the order of 15-20%. It is possible that in this condition the venous oxygen tension may be low enough to result in a generalized increase in capillary permeability. This conclusion would fit with the observations of Fine and Seligman (25, 26). They have noted that following saline therapy in hemorrhagic shock, plasma proteins are carried out of the blood stream with the saline and that this occurs to a greater extent in irreversible than in reversible shock.

These authors state their impression that "in irreversible shock the integrity of the capillaries as measured by the passage of radioactive protein may be impaired." Even in their more severe cases the venous oxygen saturation rarely fell as low as 5 vols. per cent and it would seem probable that only in the terminal phases of shock does the capillary oxygen content fall to values of 3-4 vols. per cent: that is, to levels where definite evidence of an increase in capillary permeability can be anticipated.

In their studies of the effects of anoxia on lymph production, Maurer (5) working with the cervical lymph of dogs, and Warren and Drinker (6) with that from the lungs, both demonstrated a marked increase in lymph flow when the oxygen content in the inspired air was reduced to values of 10% or less. Although they did not make direct estimations of venous oxygen tensions, it is possible to interpolate figures from the data supplied by Davies (27), concerning the mean venous oxygen tension during varying degrees of anoxic anoxia. His data suggests that the oxygen saturation of the mixed venous blood in the lungs, in their studies, was probably not less than 35% except in the cases where the oxygen percentage in the inspired air was considerably less than 10%. Adrenalin secretion may be stimulated by anoxic anoxia of this degree and there is increased lymph production

from the cervical lymphatics following the use of adrenalin (28). Although the total amount of lymph protein produced by their dogs increased, the concentration of protein in the lymph collected during the anoxic episodes decreased. This decrease is unexpected, if the cause of the increased lymph flow is an increase in permeability of the capillary wall. If, however, the increased lymph production was due in large measure to changes in the blood flow through the capillary bed, then the fall in lymph protein concentration can be more readily explained. In those cases where the oxygen content in the inspired air was less than 10% and in which the arterial oxygen saturation fell to less than 50%, it is probable that the oxygen saturation of the mixed venous blood was within the critical range of 15-25% necessary for the development of an increased capillary permeability. If our figures determined from studies using the human forearm also apply to the pulmonary capillary network, it is probable that in these cases anoxia played a direct part in the increased lymph flow.

Pochin (2) was led to his study of the relation of gross ischemia to edema formation by his observation of edema in a human limb, the circulation to which had been obstructed for 10 hours. It is possible to develop far

higher grades of recoverable anoxia in isolated parts of the body such as a limb, ear or finger, than in the entire organism which includes regions of very great sensitivity to anoxia such as the brain. Consequently, it is to be anticipated that evidence of edema formation as a result of anoxia of the capillary walls would be found in cases where the arterial blood supply to a region was grossly impaired. De Takats (29) has observed the frequent occurrence of edema in conditions such as traumatic angiospasm, acute peripheral trophoneurosis and even in Burger's disease. He emphasizes that this edema will vanish after sympathectomy or sympathetic paralysis. These procedures do not necessarily relieve any venous obstruction that may be present in such cases. This points to a relief of the anoxia of the capillary wall as a result of the improved blood flow as the probable cause of the improvement of the edema. Homans (30) has recently confirmed this opinion that the edema so frequently observed in arterial spasm is due to anoxia of the capillary wall. Oschner and De Bakey (31) in a discussion of the mechanism of development of edema in thrombophlebitis, conclude that it is not solely due to the increase in venous pressure as a result of the obstruction to the venous drainage from the part. They consider that reflex vasospasm of the arteries is a most important factor and that as a result of the vasospasm there is a relative anoxia of the

capillary endothelium with an ensuing increase in permeability. If blocking of the sympathetic pathways is effective in relieving arterial spasm, then the blood flow through the part increases the oxygen saturation in the venous blood rises and at the same time the edema decreases. It would be of interest to know the oxygen saturation of blood draining from the edematous regions before and after sympathetic paralysis in such cases.

It is known that in the liver which largely depends on venous blood for nourishment, the oxygen tension in the blood in the hepatic veins may fall to very low levels. Thus, Engel, Harrison and Long (32), and also McMichael (33), note hepatic venous oxygen saturations in the cat of 3-30% during hemorrhage. These low values were often obtained before the blood pressure had fallen significantly below its initial level. Frank, Seligman and Fine (34) have associated the susceptibility of the liver to damage in shock with this peculiarity of the blood supply. It would be of interest to know what part anoxia of the liver capillaries might play in their known high permeability to protein (10), and whether the effects of anoxia on capillary permeability to protein differ in the different regions of the body. Finally it may be noted that this work has been carried out with acute anoxia lasting for 30 minutes only. It is not known whether

prolonging the duration of the anoxia would significantly raise the critical level of oxygen tension at which capillary damage first occurs. However, the failure of Stead and Warren (8) to observe an increase in protein in the extracellular fluid in their subjects with chronic anoxia would suggest that this does not occur.

The preceding observations indicate that anoxia commences to affect capillary permeability to the point of significant protein leakage at a very low oxygen tension. The result being that it usually requires a combination of one or more of the factors, ischemia, anoxic anoxia, anemia or a great increase in tissue oxygen consumption to attain a sufficiently low level. It may be concluded that in states of uncomplicated mild anoxic anoxia there is no reason to anticipate changes in the capillary permeability of the limbs. On the other hand, in conditions such as fatal asphyxia, irreversible shock and ischemia in which the venous oxygen saturation may fall below the critical level of 15-25%, a significant increase in permeability probably occurs a few minutes after the commencement of such anoxia.

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Summary

1. A number of venous occlusion experiments of 30 minutes duration were performed with and without anoxic anoxia, and with and without adrenalin hypertension.

2. The calculated protein in the capillary filtrate was not increased until the local anoxia was of such a degree that the oxygen saturation in the venous blood draining from the arm was 15-25% or less.

3. The experiments with adrenalin hypertension suggested that ischemic anoxia and not the concomitant increase in capillary pressure is responsible for the increased amount of protein found in the filtrate resulting from the use of an arm cuff at a pressure of 80 mm. Hg.

4. In states of mild anoxia there is no reason to anticipate changes in the capillary permeability of the limbs. However, in asphyxia, irreversible shock and in severe local ischemia, or other states in which the venous oxygen saturation may fall below a critical level of 15-25% a significant increase in permeability to protein probably occurs a few minutes after the commencement of such anoxia.

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Table I

Fluid loss and per cent protein in filtrate with 80 mm. Hg. congestion (controls)

Subject	Hematocrit	Fluid loss cc/100 cc. (Hematocrt)	Fluid loss cc/100 cc. (Hemoglob)	Grams Protein	% Protein in Filtrate	% Saturation of Venous Blood
5c	43.4 45.8	5.6	3.9	6.55 7.10	+ 1.5	
5d	41.9 46.9	10.9	8.6	6.19 7.37	+ 1.0	21.6
5e	40.7 46.1	11.7	10.7	5.76 6.93	+ 1.0	21.3
7q	41.3 47.2	12.6	18.2	6.76 7.79	+ 3.0	18.9
7r	42.8 52.7	18.9	19.1	6.11 8.88	+ 0.5	19.1
7s	43.0 52.6	18.3	17.5	5.90 8.66	+ 0.1	15.6
6j	39.5 43.5	9.4	6.6	5.97 7.27	- 1.1	18.2
6k	39.6 49.6	20.1	17.0	6.14 7.82	+ 2.8	16.9
6l	41.0 47.2	13.2	13.9	5.80 7.92	- 1.6	12.1
Average		13.4	12.8		+ 0.8	18.0
Standard deviation of mean		± 1.7	± 1.8		± 0.5	

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Appendix III

Table II

Fluid loss and per cent protein in filtrate with 80 mm. Hg. congestion (Adrenalin hypertention)

Subject	Hematocrit	Fluid Loss cc/100 cc. (Hematcrt)	Fluid Loss cc/100 cc. (Hemoglob)	Grams Protein	% Protein in Filtrate	% Saturation of Venous Blood
5f	40.1 42.1	4.9	3.9	5.42 6.17	- 3.0	24.3
5g	40.0 44.0	9.2	10.4	5.97 7.07	- 0.1	27.8
7t	44.2 47.4	6.8	4.8	6.17 7.24	- 1.5	42.3
7u	44.2 48.3	8.5	7.0	6.35 7.58	- 0.5	35.4
7v	44.8 46.5	3.6	4.9	6.52 7.03	- 0.8	51.9
6m	42.8 45.2	5.1	5.2	6.48 7.03	+ 0.9	19.5
6n	43.5 47.2	8.1	7.9	6.93 8.23	- 0.8	14.3
6o	42.5 46.2	7.9	8.9	5.93 7.27	- 2.5	28.4
Average		6.0	6.6		- 1.0	30.5
Standard deviation of mean		± 0.6	± 0.8		± 0.3	

Appendix III

Table III

Fluid loss and per cent protein in filtrate with 60 mm. Hg. congestion: controls

Subject	Hematocrit	Fluid Loss cc/100 cc (Hematcrt)	Fluid Loss cc/100 cc (Hemoglob)	Grams Protein	% Protein in Filtrate	% Saturation of Venous Blood
1a	40.4/23.2	5.9	8.5	7.05/8.10	- 2.5	
1b	41.4/43.8	4.5	5.1	7.30/8.03	- 1.5	
1c	40.7/42.5	4.3	1.5	6.88/7.23	+ 2.5	
2a	38.6/42.4	9.1	6.7	6.94/8.10	+ 0.3	
2b	39.2/43.0	8.7	7.4	6.68/7.75	+ 0.3	
3a	38.3/41.2	7.1	4.0	6.50/7.03	+ 2.4	
3b	41.7/43.4	3.6	3.0	6.90/7.30	+ 0.8	
4a	43.0/45.7	5.8	-	6.46/7.28	- 0.7	
5a	42.8/45.9	6.8	2.0	6.04/6.79	+ 0.5	28.7
5b	41.0/43.0	4.7	6.3	5.52/6.11	- 1.3	33.5
6a	41.5/47.1	12.0	12.1	6.48/7.31	+ 3.3	13.3
6b	40.9/43.6	6.1	9.6	6.17/6.86	+ 0.2	17.2
6c	41.2/45.7	10.0	9.4	6.04/6.93	+ 2.2	23.0
7a	42.5/46.7	8.8	13.9	6.27/7.28	+ 0.6	
7b	42.1/47.0	10.5	12.4	6.53/7.75	+ 1.0	
7c	44.2/48.2	8.4	3.1	6.45/7.61	- 0.1	
7d	46.2/49.8	7.3	-	6.14/7.34	- 1.5	
7e	46.0/48.6	5.3	5.1	7.00/7.51	+ 2.3	
7f	41.5/44.7	7.4	6.9	6.07/7.03	- 0.6	
7g	42.4/47.1	10.1	20.3	6.55/7.65	+ 1.4	
7h	41.4/46.3	10.7	12.6	5.83/7.44	- 1.4	25.7
7i	42.5/47.1	9.7	13.2	5.52/6.79	- 0.7	43.1
6d	39.5/42.6	7.3	9.9	6.14/6.72	+ 1.9	
Average		7.6	8.2		+ 0.4	
Standard Deviation of mean		± 0.5	± 1.1		± 0.3	26.4

Fluid loss and per cent protein in filtrate with 60 mm. Hg. congestion
(Arterial Saturation 55-65%)

Subject	Hematocrit	Fluid Loss cc/100 cc (Hematcrt)	Fluid Loss cc/100 cc (Hemoglob)	Grams Protein	% Protein in Filtrate	% Saturation of Venous Blood
7j	47.2 49.4	5.6	5.6	6.83 7.41	+ 1.9	
7k	48.8 51.7	5.7	6.3	6.96 7.75	+ 0.7	
7l	44.2 48.6	9.2	6.4	6.79 8.06	+ 0.4	
7m	43.6 47.6	8.3	11.0	7.34 8.30	+ 1.8	
7n	43.8 46.8	6.5	8.1	6.21 6.72	+ 2.3	11.7
7c	45.7 48.7	6.0	3.2	6.04 6.52	+ 2.2	13.0
6e	41.4 44.5	7.0	3.9	6.41 7.48	- 1.5	
6f	38.6 41.9	7.9	5.4	6.07 6.55	+ 2.8	
6g	42.8 47.5	9.6	9.7	5.80 6.89	+ 0.4	11.0
6h	42.7 47.4	9.9	8.2	6.04 7.10	+ 1.2	8.9
7p	44.9 45.8	2.0	0.6	7.00 7.13	+ 3.6	14.8
6i	41.5 42.5	2.4	0.8	5.48 5.76	- 1.0	9.2
Average		6.7	5.8		+ 1.2	11.4
Standard Dev. of mean		± 0.7	± 1.0		± 0.4	

Engineering Division
Memorandum Report No. MCREXD-696-104D
18 February 1948

Appendix III

Table V

Summary of changes obtained in fluid loss and per cent protein in the filtrate when anoxia is added to a 60 mm. Hg. congestion and adrenalin hypertension to an 80 mm. Hg. congestion experiment.

	Values based on changes in Hematocrits	Values based on changes in Hemoglobins	Values based on changes in Hematocrits	Values based on changes in Hemoglobins
	60 mm. Hg. controls (23 expts)		60 mm. Hg. with anoxia (12 expts)	
Mean Fluid Loss	7.6 cc/100 cc	8.2 cc/100 cc	6.7 cc/100 cc	5.8 cc/100 cc
Standard Deviation	± 0.5 cc	± 1.1 cc	± 0.7 cc	± 1.0 cc
Mean Protein in Filtrate	- 0.4 gms %		+ 1.2 gms %	
Standard Deviation	± 0.3 gms %		± 0.4 gms %	
Mean Venous O_2 Saturation	(26.4 %)		(11.4%)	
	80 mm. Hg. control (9 expts)		80 mm. Hg. with adrenalin (9 expts)	
Mean Fluid loss	13.4 cc/100 cc	12.8 cc/100 cc	6.0 cc/100 cc	6.6 cc/100 cc
Standard Deviation	± 1.7 cc	± 1.8 cc	± 0.6 cc	± 0.8 cc
Mean Protein in Filtrate	+ 0.8 gms %		- 1.0 gms %	
Standard Deviation	± 0.5 gms %		± 0.3 gms %	
Mean Venous O_2 Saturation	18.0 %		30.5 %	

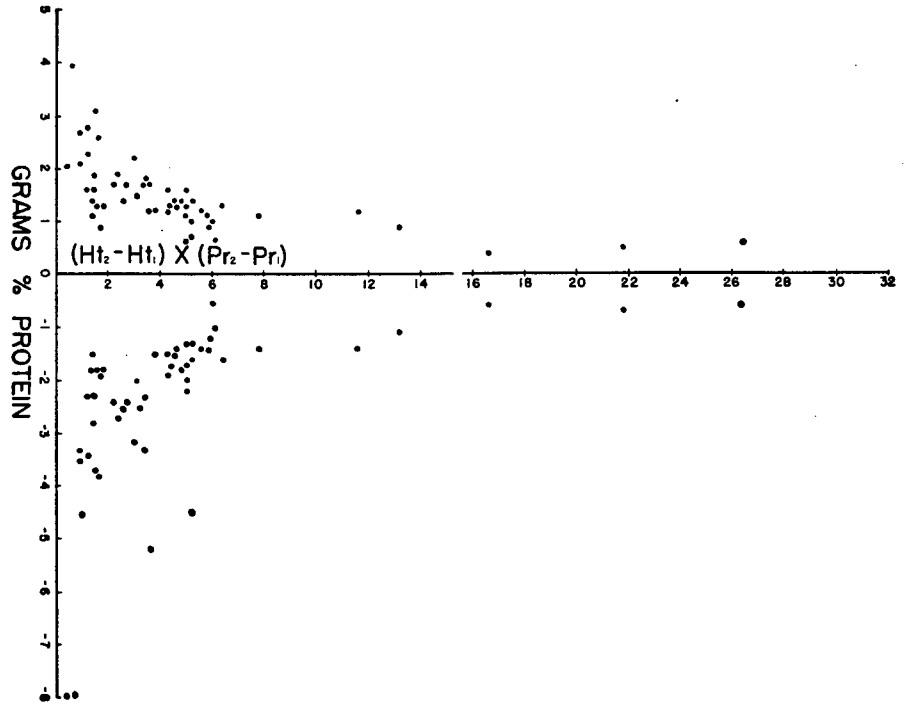

Figure 1. (Appendix III)

Variability in calculated percent protein in the filtrate as a function of the changes in Hematocrit and Protein values.

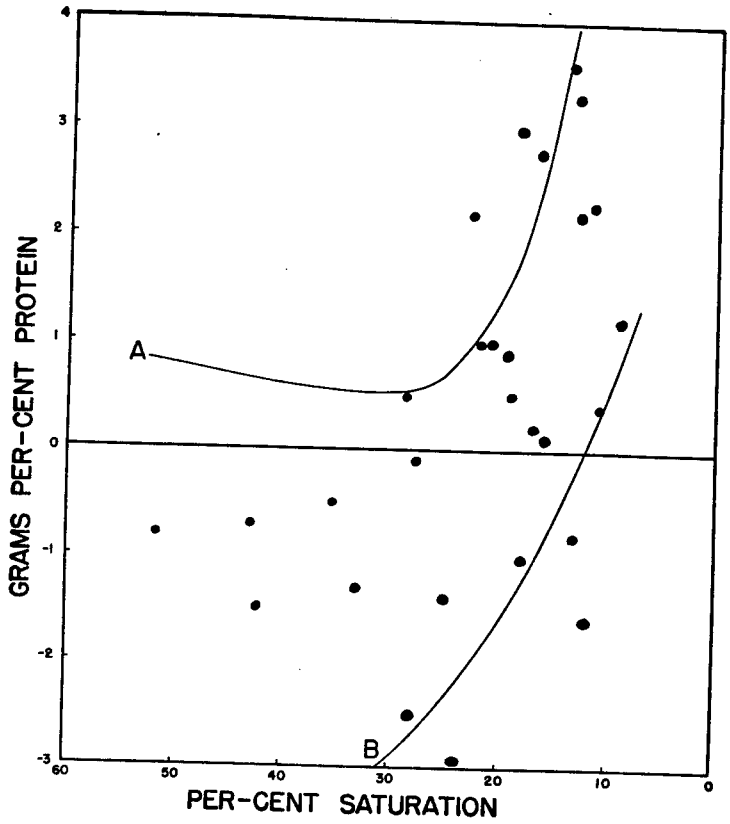

Figure 2. . (Appendix III)

Percent protein in filtrate as a function of the venous oxygen saturation.

Engineering Division
Memorandum Report No. MCREXD-696-104D
18 February 1948

Appendix IV

Method for Perfusion of Isolated Rat Tissues

Chester Hyman and R. W. Bedan

Apparatus:

Fig. 1 is a photograph of the apparatus used in the perfusion of rat tissues with continuous recording of the weight. The perfusion system is essentially the same as described by Hyman and Chambers, with two modifications: The all-glass perfusion reservoirs were replaced by ordinary Mason jars (P.R.) with holes and compression fittings mounted on their covers; the pressure regulatory system was revised to include an electrically controlled valve in place of the mercury blow-off. In the firgure, (S) houses a pressure actuated switch which controls the pressure regulating valve (V).

The perfused tissues are placed in a moist chamber (M) at one end of a sensitive balance. The other arm of the balance carries a pan (C.W.) for counter weights and a phosphorbronze spring, (Sp). This system permits use of a more sensitive spring by balancing out most of the dead weight. The perfusion fluid is conducted to the balance arm through rubber tubing at the exact axis of rotation, thus providing minimum torque.

Recording is accomplished by periodic discharge (two second discharge followed by a 13 second rest interval) of an induction coil between a pin at the end of the level arm (W.P.) and a metal strip (S.T.) behind a slowly moving waxed recording paper. This method of recording provides an effective, friction-free system.

The rate of flow through the preparation is followed by catching the outflow in a calibrated tipple (T) which activates a signal magnet (SM) writing directly on the record.

Results:

In these studies preparations were made from the isolated hind limbs of a rat and were perfused with a uniform solution of oxy-poly-gelatin (6) in saline. The solution was placed in equilibrium with gas mixtures containing varying amounts of oxygen. No attempt was made to introduce red cells, hemoglobin or any other agency for oxygen transport into the perfusion fluid. Such preparations will display a uniform rate of edema formation for periods up to four hours if all conditions are held constant. When the oxygen content is reduced to levels below 10% of the total gas mixture, a marked increase in the rate of edema formation follows.

The rate of protein leakage was estimated by a modification of this technique. The rate of edema formation

is measured in a similar preparation during perfusion with a colloid-free saline solution. This is followed by perfusion with gelatin for 30 minutes, after which perfusion with saline is restored. Differences in the rate of edema formation between the first and second saline perfusions measure the accumulation of colloidal material in the extra-vascular spaces. Experiments of this type performed with both saline and gelatin solution in equilibrium with varying gas mixtures show that protein leakage is increased at oxygen concentrations below 10%. These results are summarized in Table I.

One further experiment with perfused tissues was made to assess the significance of stagnation in altering capillary permeability. Routine perfusions using gelatin solution were interrupted for varying periods of time, subjecting the tissue to stagnant anoxia. Periods of stagnation up to 15 minutes had no effect on the subsequent rates of edema formation, whereas longer periods uniformly led to measurable increases in edema. The arteriolar tone in these preparations, measured by flow rates at constant pressure, was maintained during periods of stagnant anoxia up to 15 minutes, but longer periods produced a relaxation or at least, an increased flow rate. The present data do not permit differentiation between alteration in circulatory dynamics as suggested by these flow data and a direct effect of the anoxia on the capillary wall.

Appendix IV

Table I

Rate of Accumulation of Colloid in Perfused Anoxic Tissue

% O in Equilibrium with Soln.	Number of Experiments	Sf as * % Si
20%	8	180
10%	7	380
0%	5	400

* Sf as % Si is the ratio of the edema rate measured during the final perfusion with saline to the edema rate measured during the initial perfusion with saline. In the intervening period the animal had been perfused for one hour with gelatin.

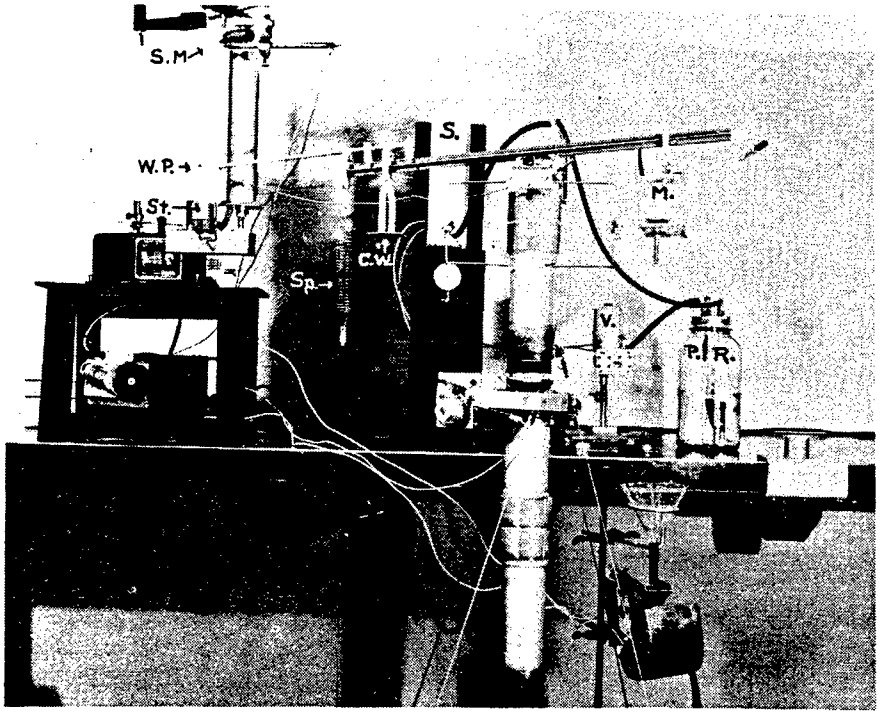

Figure 1. (Appendix IV)

Photograph of apparatus for continuous recording of weight of perfused preparation. For Key, see text.

Engineering Division
Memorandum Report No. MCREXD-696-104D
18 February 1948

Appendix V

The Effects of Temperature and Exercise on Venous Pressure in the Foot When in the Erect Posture

James P. Henry

The changes occurring in the circulation following the assumption of the erect posture clearly show the influence of gravity upon the vascular system. Chapman and Asmussen (1) and others have described the rapid pooling of blood in the legs and the fainting that follows shift of the longitudinal axis of the body from the horizontal to the vertical position by use of the tilt table. Mayerson and Burch (2) and others (3, 4, 5 and 6) have shown that if the subject stands with the legs relaxed, this pooling is accompanied by the development of a venous pressure in the feet which is equal to the full hydrostatic head of pressure from heart to foot. However, they note that on contracting the leg muscles, as by raising the body onto tiptoe, the venous pressure becomes significantly reduced (2). There are many valves in the deep veins of the calves and those in the communicating veins between the deep and superficial plexuses operate to check the flow of blood superficially (7 and 8). Mayerson and Burch (2) and others (7 and 9) have also shown that the high pressure generated within the contracting muscle are more than enough to force the blood out of the lower segment of the leg, past the knee,

into the upper part. In the act of walking the contracting thigh drives the blood on into the abdominal cavity. Beecher, Field and Krogh (7) have used a capsule device for indirectly measuring venous pressure to show that when walking, the mean venous pressure at the foot is probably less than the colloid osmotic pressure of the blood and thus, edema formation was not to be expected in the normal person when erect and moving. However, when the subject is still, the venous pressure rises to the fully hydrostatic head and exceeds the colloidal osmotic pressure.

It was observed during the course of observations of venous pressure in the erect posture, in this Laboratory, that direct measurements of pressure during exercise gave variable and unpredictable values. Control of the temperature of the foot under observation increased their consistency and measurements were, therefore, made of the mean venous pressure with gentle exercise at two different temperatures in order to define more clearly the effect of temperature on mean venous pressure when exercising.

Methods:

Ten experiments were made using eight subjects, all of whom were healthy males without vascular abnormalities (10) between the ages of 24 and 33. Vein puncture was performed

by use of an Ungar type double needle with a sharp inner component (11).

Removal of the inner needle left a blunt outer cannula which was connected by a three-way stopcock to a rubber jointed sterile glass tube extending up to a shoulder level. This type contained sterile heparinsed physiological saline. The temperature of the foot was controlled by immersion up to the ankle in a water bath either at 104° to 113° F, or at 65° to 70° F. Room temperature was relatively constant and averaged 72°. Single Van Slyke blood gas determination of oxygen content were carried out on samples taken from the cannula.

A typical experiment was carried out in the following manner: The subject was seated and his foot immersed at 100° F for 10 minutes until the superficial veins of the foot dilated fully. He then stood up, placed his foot on the edge of the bath, the double needle was introduced into a dorsal vein of the foot, the inner component removed and the three-way stopcock was attached. Sufficient saline was introduced into the manometer tube to reach above the heart level and the foot was again immersed in the bath. After a few seconds of quiet standing, the fluid level would come to

rest approximately opposite to the third or fourth rib. The subject then performed standing - walking by shifting the weight from one foot to the other without raising the ball of the foot from the bottom of the bath. The fluid level in the manometer sank progressively and after two or three minutes became stable. After measuring the height of this column, a venous sample was taken, the three-way stopcock was removed, the stilette inserted and the bath temperature changed to 65° to 70° F. Both feet were placed in this water to accelerate the rate of loss of body heat with consequent vasoconstriction, and the skin of the immersed feet was watched for 15 to 30 minutes until slight cyanosis developed. The resting venous pressure was then measured as before and the lowest level of venous pressure attained was noted. A blood sample was taken, the needle removed and firm pressure applied to the puncture site for 5 minutes with the foot elevated.

Results:

In Table I the mean values obtained for ten experiments carried out in the hot bath are presented together with those found in the cold. In both cases when standing still, the full hydrostatic head from heart to

foot was attained. The small difference between the mean level when cool (125 cms.) and the slightly higher value when hot (129 cms.) may be significant. It may be due to the impossibility of keeping quite free from all muscular movement when standing erect (3), and an expression of the greater effect on venous pressure of such movements when the blood flow is reduced. The temperature of the cool bath was approximately that of the room. Sheard, Williams and Horton (12) have noted that the skin temperature of the feet is very close to room temperature when this is in the range used for these studies. In Table I it is shown that the respective values for immobility and exercise are 125 cms. and 52 cms. It may be noted that similar results can be obtained without a cool foot bath and using 9 subjects at a room temperature of 70° to 75° F, a mean value for the venous pressure of 133 cms. was obtained when standing still and 60 cms. when exercising, using the standing - walking technique. These values roughly correspond to "heart" level and to the level of the "knee" in the average subject.

During exercise higher figures were consistently obtained for the mean venous pressure in the warm bath than at the lower temperature. Thus, in Table I the mean pressure

when still was 129 cms. and when exercising, it was 96 cms. This later value corresponds to a venous column extending up to the inguinal region. The contrast between the mean venous oxygen content of 16.2 vols. per cent at 104° to 113° F, and of 9.7 vols. per cent at 65° to 70° F is an indication of the marked speeding up of the blood flow which has occurred in every case. The values in the cold are similar to those found by Goldschmidt and Light at environmental temperatures of 60° to 70° F (13). The venous oxygen content, during heating, more closely approaches the values they obtained at 80° to 90° F. If it is assumed that the oxygen content of equilibrated blood is approximately 20.7 vols. per cent (14), then the difference in the amount of oxygen removed from the blood in the warm foot is (20.7 to 16.2, i.e. 4.5 vols. per cent) in contrast with that from the cool foot, (20.7 to 10.0, i.e. 10.7 vols. per cent). This suggests that at least twice as much blood is flowing through the veins in the heated as in the cool foot. It is of significance in this connection that Freeman in his comparative studies of blood flow in the normal human hand, when cool and when hot, found a difference of rate of blood flow in the two conditions of the order of one to ten (15 and 16).

Discussion

The results contrasting venous pressure during movement with that when still, confirm those obtained by previous workers (2, 3, 4, 5, 6 and 17) who, in the absence of specific reference to this point, may also be presumed to have worked at room temperatures of from 65° to 75° F. Several authors have pointed out that the high incidence of edema of the ankles in patients in catatonic states and after prefrontal lobotome (18) in the aged and in convalescence (19), may probably be attributed in part to immobility. It is also of interest that Smirk (6) considers that a significant part is played by immobility in the development of edema of the ankels in cardiac cases. There are cases in which the factors of impaired renal function (20) with sodium retention (21), decreased osmotic pressure of blood (6) and increased central venous pressure do not seem sufficiently severe to explain the development of edema. In such cases the suggestion that the mean pressure in the peripheral veins is increased (6) and contributes to the edema formation becomes of considerable interest.

The mechanism of development of the dependent so commonly observed in hot weather when the environmental

temperature is in the range of 85° to 105° F (22), may be related to the observations made in this report. Under conditions of high environmental temperature, there is a much greater severity of edema of the ankles in thos normally predisposed; that is, in cardiac cases (23), persons with obesity and in those suffering from varicose veins with incompetent communicating channels. In addition, edema may occur in such weather in normal persons who may be merely sitting or standing still for prolonged periods. Thus, Hardy, Milhorat and Dubois note the case of a normal woman who developed marked vasodilation of the feet in hot weather and with it, edema of the ankles (24). In hot weather vasodilation of the vessels in the feet commonly occurs when the temperature is greater than 80° to 85° F (13). The exact temperature at which this dilation develops will depend upon the individual (25). It is assumed that this vasodilation leads to an overloading of the capacity of the muscular pump, which can remove only a certain amount of blood and lymph for a given amount of muscular activity. In addition, in hot weather such activity often decreases. Therefore, while the cutaneous blood flow rate may increase by a factor of 2 to 5 times, the rate of pumping of blood

and lymph may actually decrease. As a result, the mean venous pressure may rise significantly above the normal level and edema develop.

Summary

1. The venous pressure in the foot in the erect posture is dependent upon the environmental temperature as well as upon the activity of the limb.

2. When completely still, the venous pressure attains the full hydrostatic head regardless of the environmental temperature.

3. Standing - walking at an environmental temperature of 65° to 75° F will lead to a mean venous pressure at or less than knee level.

4. The same exercise when the foot temperature is maintained at 104° to 113° F will produce a mean venous pressure which supports a blood column extending up to the inguinal level.

5. "Heat edema" may be due in part to this increase in mean venous pressure.

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Appendix V

Table I

Venous Pressure when in the erect Posture under Varying Conditions of Exercise and Temperature

			T 104° to 113° F			T 65° to 70° F		
Date	Sub.	Exp.	Still	Exercise	Venous O_2 Vols. %	Still	Exercise	Venous O_2 Vols. %
5/7/47	JH	1	124 cms.	90 cms.	----	112 cms.	51 cms.	----
5/8/47	DD	2	150	102	17.7	144	33	----
5/9/47	JG	3	123	94	14.4	123	61	8.0
5/13/47	DD	4a	135	99	----	119	38	----
		4b	145	102	----			
5/21/47	JH	5a	123	97	17.8	121	46	10.0
		5b	122	94				
5/22/47	LD	6	135	112	----	132	64	----
5/26/47	BC	7	126	91	17.2	125	51	9.4
5/27/47	CI	8a	122	97	16.8	122	66	12.7
		8b	122	91				
5/28/47	JG	9a	122	81	16.3	122	53	9.5
		9b	122	89				
5/29/47	RI	10	131	99	15.9	130	61	8.7
Average			mean 129 cms.	mean 96 cms.	16.3 vols.	125 cms.	52 cms.	9.7 vols.
Approximate levels on average subject			"Heart Level"	"Inguinal region"			"Knee Level"	

Engineering Division
Memorandum Report No. MCREXD-696-104D
18 February 1948

Appendix VI

Production of Polycythemia in Rabbits by Anoxia and Cobalt

Joseph Goodman and Byron Howells

A need for polycythemic rabbits as experimental animals led to the consideration of methods of producing the state of polycythemia in this species. There are at least two experimental methods for inducing this condition. The older and better known of these is the response to anoxia induced by high altitude; another is the administration of cobalt salts.

Although the stimulating effect of lowered oxygen tension is well known and has been extensively studied, its mechanism is not as yet clearly understood. The effect has been noted in many animals other than man (1). These include monkeys (2), dogs (3), rats (3) and rabbits (3 and 5).

Armstrong and Heim (4 and 5) reported on the exposure of young male rabbits for 4 hours daily 5 days a week to pressures equivalent to 18,000 feet. In addition to this treatment the animals were subjected to an altitude tolerance test on the 7th day. This tolerance test involved rapid decompression to the point where the animals became unconscious.

The equivalent altitude attained was noted and the animal immediately returned to ground level. In the 3rd week of their experiment a deterioration set in and the hemoglobin and hematocrit levels depreciated to lower than the original values. They concluded that rabbits were quite unsatisfactory for this work.

Later Thorn, Jones, Lewis, Mitchell and Koepf (3) conducted a series of experiments in which they exposed rabbits to pressures equivalent to 18,000 and 25,000 feet for 4 hours a day 7 days a week. The 18,000-foot animals were continued for 5 weeks whereas the 25,000-foot experiment was discontinued at the end of 3 weeks because of excessive fatalities. Those exposed to 18,000 feet showed a temporary delay in growth, together with a slight drop in the concentration of serum, sodium chloride and CO_2 combining power and a decrease in the weight of the thymus gland. These changes were accompanied by an increase in O_2 capacity of the blood, in the hematocrit values and in the adrenal gland weight. Seventy-five percent of those exposed to 25,000 feet died of hemorrhage into the lungs and/or herniation of distended loops of the intestines into the thoracic cavity. There were were no fatalities in the 18,000-foot group, nor was there evidence of degeneration in the hemopoietic system.

It may be noted that little is known of the mechanism or the optimum conditions for producing polycythemia by this method.

Another technic for producing polycythemia, whose mechanism is, however, even less clearly understood, was reported by Waltner and Waltner (6). They induced polycythemia by the administration of cobalt. This method has been used on several species but was found to be slow and sometimes accompanied by toxic symptoms. Frost et al. (7) produced a temporary polycythemia in adult dogs by including cobalt in the food. However, they found this toxic to young growing dogs. On the other hand, Stanley, Hopps and Hellbaum (8) reported the development of polycythemia in rats by a subcutaneous injection without unfavorable symptoms.

Methods:

It was decided to study the possibilities of both the above mentioned technics for producing experimental polycythemia. The routine of the cobalt injections was patterned after that employed by Stanley et a. (8). The exposure time at altitude was materially increased above the time used by previous workers, but remained intermittent. This allowed the animals some time for recuperation under more nearly optimum living conditions at sea level. Altitude

and cobalt stimuli were tried separately and together on immature male rabbits. Twelve animals, averaging 3½ lbs. were divided into 4 groups of 3 rabbits each. Group I served as controls remaining at sea level pressures and without cobalt injections. Group II was subjected to an ambient pressure equivalent to an altitude of 20,000 feet, 16 hours a day, 5 days a week. Group III was injected subcutaneously with 1 ml. of sterile cobalt solution per day 6 days a week, but remained at ground level (300 feet above sea level). Group IV was injected subcutaneously with 1 ml. of cobalt solution per day 6 days a week and was subjected to a pressure equivalent to 20,000-foot altitude 16 hours a day, 5 days a week. The cobalt solution consisted of 10 mg. $CoCl_2{:}6H_2O$ per cc. made up to isotonicity with NaCl. This was injected subcutaneously into a shaved area of the back and shoulders. The altitude was simulated in a decompression chamber. The altitude animals rested at ground levels (300 feet) each day for 8 hours and over Saturday and Sunday night, a period of 56 hours. The altitude "flights" were continued for 10 weeks. This was followed by a 2½-week ground level observation period.

The animals were weighed and sampled twice a week, on Mondays at the end of the 56-hour sea level resting period

and on Thursdays after 3 of the 5 successive night flights of 16 hours. The blood samples were taken from the marginal ear vein. Hemoglobin was determined by the acid hematin method with a Klett Summerson colorimeter. Hematocrit values were measured in Wintrobe tubes. Plasma volume was determined by the dye method using T-1824 (9). The plasma volume was measured only 4 times during the course of the entire experiment. These were, first during the pre-stimulus period, next after 3 weeks of exposure to altitude and/or cobalt, then after 10 weeks of stimuli, and lastly at the end of the post-stimuli observation period. The dye determination technic consisted of the injection of a weighed amount of 1% dye solution in isotonic saline. After 8 minutes a blood sample was taken, using 1.6% sodium oxalate solution as an anticoagulant. The blood sample-oxalate ratio was determined in each case by weighing and the ratio used to correct the readings obtained. The intensity of the blue color in the plasma was measured on a Beckman quartz spectrophotometer, using the 620 m absorption band.

Results:

The groups of animals employed were too small to permit satisfactory statistical analysis. However, the response to the stimuli was definite and conclusively demonstrates the possibility of producing polycythemia in this species without deterioration.

Fig. 1 shows the average of the weight, hemoglobin and hematocrit values of the control group. (Only 2 animals are reported in this group. One was stolen on the second week of the experiment.)

The response to altitude alone is shown in Fig. 2. Each parameter represents the average value for 3 animals. (No fatalities.) The exposure to altitude prompted a decrease in weight, followed by a period of slow recovery with a final resumption of a growth rate equivalent to that of the controls. Hemoglobin and hematocrit values both showed a rapid increase during the weekend resting period after the first exposures to altitude. This sloped off; first into a period of little change and then into a gradual climb which persisted as long as the stimulus was applied. The decrease in hemoglobin and hematocrit from the 25th to 35th day coincided with a similar depression in the control group and a period of high summer heat. It is to be noted that, during the control period following the altitude exposure the hemoglobin and hematocrit values returned rapidly toward the normal levels.

One of the cobalt-injected, ground level group, died apparently from the toxic effects of the cobalt combined with severe anorexia. The data in Fig. 3 represent the average of the values obtained from the 2 remaining animals.

The weight curve generally follows that of the controls. The hemoglobin value dropped slightly when the injection started, followed by a gradual increase for the period of the injections, but did not attain values comparable to those in the animals subjected to altitude. The return to normal, after injections were stopped, was slower than that found with the altitude rabbits.

Two of the 3 animals receiving combined altitude and cobalt treatment died; one apparently from the toxic effects of the cobalt, the other from the combination of toxic effects and peritonitis. Sterile abscesses developed on all 6 animals receiving cobalt injections at or near the site of the injection. The larger of these were opened and drained; however, no infections developed in these areas. The curves for the one surviving altitude and cobalt animal are shown in Fig. 4. They closely resemble those found in the Group II, or altitude only animals, with the exception of the absence of the depression of hemoglobin and hematocrit values from 25th to 35th day. This may be due to individual variation. The differences are not sufficient to indicate that the 2 stimuli are additive in producing polycythemia. However, a more prolonged experiment might show this to be true.

Engineering Division
Memorandum Report No. MCREXD-696-104D
18 February 1948

The plasma volume and the whole blood volume data are given in Table I, as the average of each group in per cent increase above the pre-stimulus period. In this manner each animal is compared to himself at the start of the experiment, and as all the animals were still growing all values are positive. The significance can then be shown by comparing the various groups to the control group. The whole blood volume values were obtained from the plasma volume and the corresponding hematocrit value.

The values in parentheses show the per cent change found during each period (i.e. the increase or decrease in percentage of each period as compared with that of the preceding period). From this one can more easily follow the progressive changes at each period of the experiment.

After 3 weeks of stimuli (Table I, Column 4) there was no significant increase in whole blood volume above that of the controls. However, the hematocrits of those exposed to altitude showed an increased 45% in red cell volume at the expense of the normal increase in plasma volume. The animals exposed to altitude showed almost no change in the plasma volume (3% as compared to 50% for the controls, Table I, Column 1).

During the ensuing 7 weeks of exposure to stimuli,

increase of plasma volume in the experimental animals was comparable to the increase in the controls (from 22.5% increase to 32.1% increase). At the same time, the increase in the whole blood volume of the altitude animals (66% and 68%) was more than double the increase of the controls (24.2%). This suggests that the development of polycythemia may have two phases or processes; an initial immediate and rapid one involving an increase in the cellular phase at the expense of the plasma volume and a second more gradual step during which the plasma volume increases at a normal rate together with a further increase in the red cell volume.

The changes occuring after removal of the stimuli are, for the most part, in the red cell component of the blood. The plasma volume of the altitude animals increased above that of the controls, and there was a large drop in the whole blood volume.

Even after 10 weeks of cobalt injections, Group III did not have a greater whole blood volume than that of the controls. However, they showed only a moderate polycythemia which was attained at the expense of the plasma volume. Yet after the stimulus had been stopped, there was a decrease in plasma volume as well as total red cell volume.

This either indicates that the development of cobalt and altitude polycythemia involves different mechanisms of response, or that the cobalt animals at 10 weeks had only developed to a stage comparable to the first 3 weeks of altitude exposure.

Conclusion:

Polycythemia can be rapidly induced in rabbits by exposure to altitudes of 20,000 feet 16 hours a day, 5 days a week. It would appear that there is no additive effect when cobalt injections are combined with the altitude exposure.

There may be two stages in the development of polycythemia. The first phase is characterized by a normal whole blood volume with a subnormal plasma volume. The second phase shows a normal rate of increase in plasma volume with a further increase in whole blood volume.

Subcutaneous cobalt injections slowly induce polycythemia in rabbits but they are accompanied by anorexia, sterile abscesses and other symptoms of toxic effect.

Engineering Division
Memorandum Report No. MCREXD-696-104D
18 February 1948

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Appendix VI

Table I

Percent Increase Above the Pre-experimental Value

	Plasma volume			Whole blood volume		
	After 3 weeks of stimulus	10 wks of stimulus	2½ wks of recovery	After 3 wks of stimulus	10 wks of stimulus	2½ wks of recovery
Column No.	1	2	3	4	5	6
Control	+ 50.0%	+72.5% (+22.5) *	+ 86.0% (+ 13.5)	+ 39.6%	+ 63.8% (+ 24.2)	+ 74.5% (+ 10.7)
Cobalt and altitude	+ 3.3	+28.0 (+24.7)	+ 33.0 (+ 5.0)	+ 40.4	+107.0 (+ 66.6)	+ 60.0 (- 47.0)
Altitude	+ 2.6	+34.7 (+32.1)	+ 57.7 (+23.0)	+ 33.7	+102.2 (+ 68.5	+ 69.3 (- 32.9)
Cobalt	+ 12.3	+40.5 (+ 28.2)	+33.5 (- 7.0)	+ 26.0	+ 63.3 (+ 37.3	+ 40.0 (-23.3)

* Values in parentheses show the changes in percent increase above the values of the previous period.

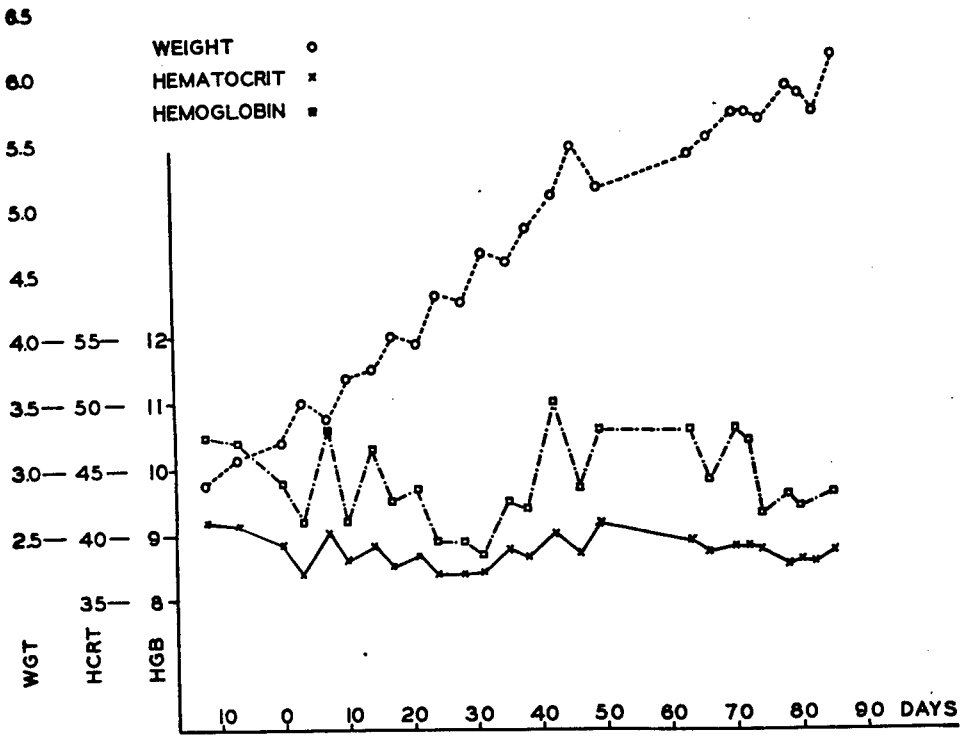

FIGURE 1 SEA LEVEL CONTROL

Figure 1. (Appendix VI)

Hemoglobin, hematocrit and weight curves for sea level control animals.

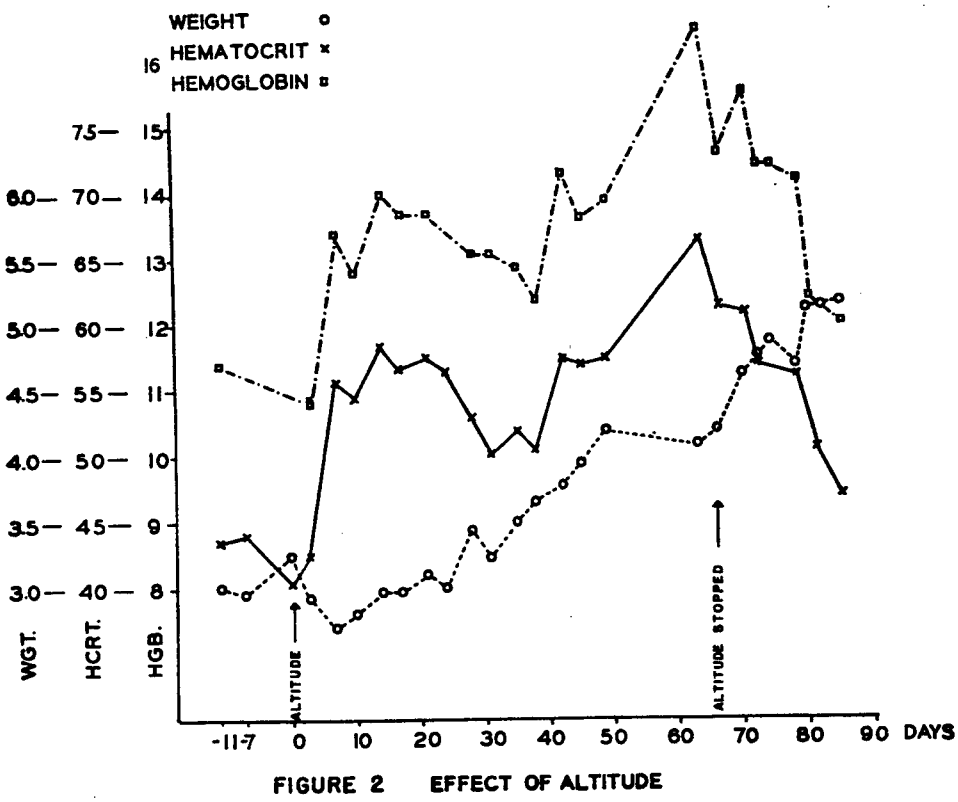

FIGURE 2 EFFECT OF ALTITUDE

Figure 2. (Appendix VI)

Hemoglobin, hematocrit and weight changes in animals exposed to diminished ambient pressures.

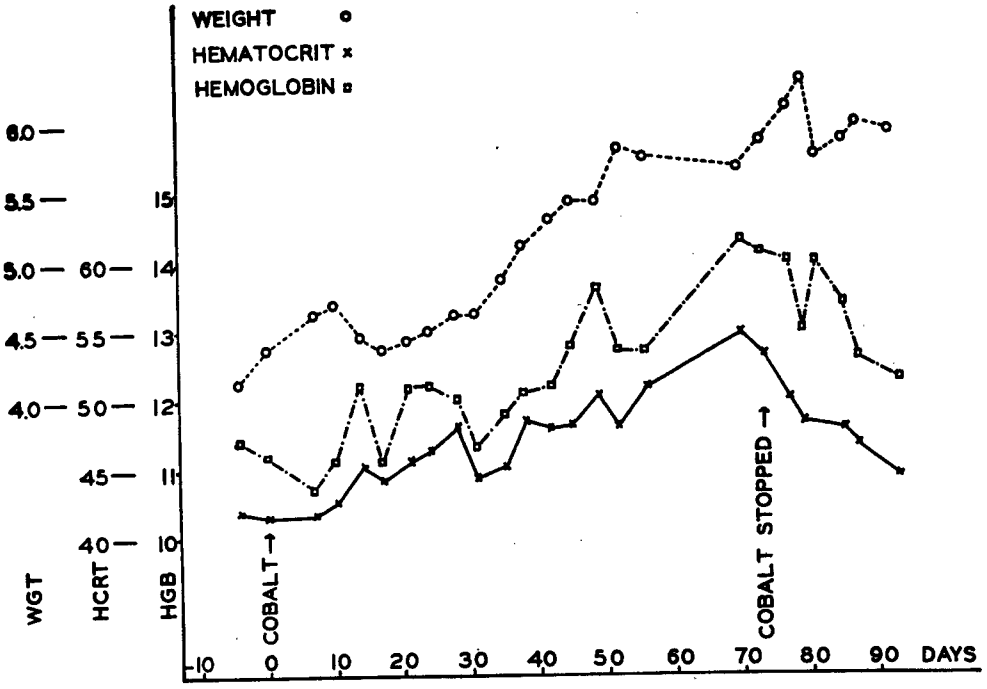

FIGURE 3 EFFECT OF COBALT

Figure 3. (Appendix VI)

Hemoglobin, hematocrit and weight changes in animals injected with cobalt.

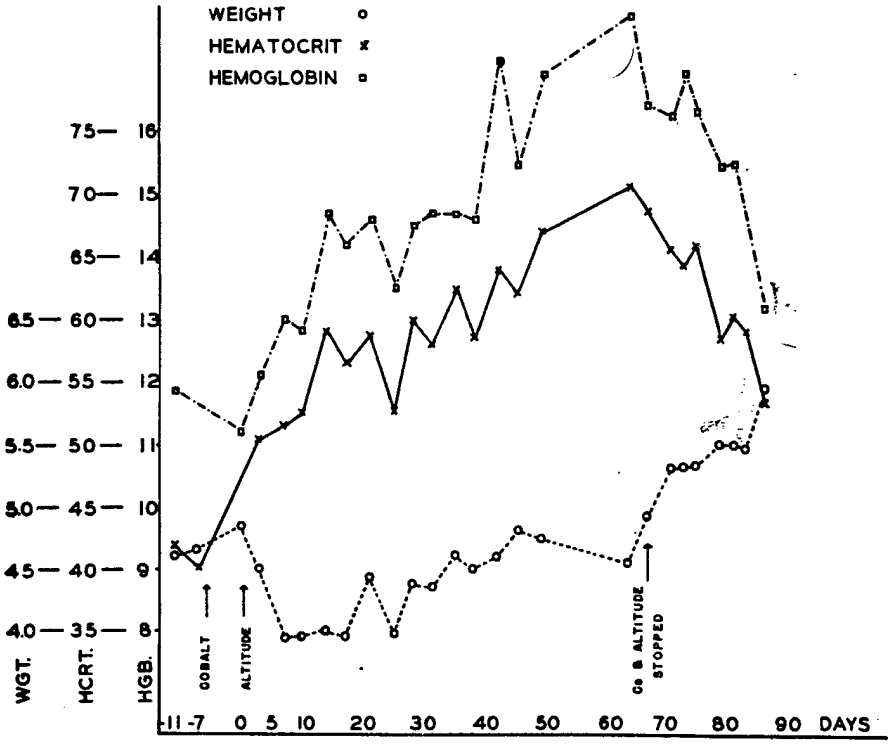

FIGURE 4 EFFECT OF COBALT AND ALTITUDE

Figure 4. (Appendix VI)

Hemoglobin, hematocrit and weight changes in animals injected with cobalt and exposed to diminished pressures.